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EXPERIMENTAL INVESTIGATIONS ON THE GENUS RAZOUMOFSKYA

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(WITH NINETEEN FIGURES)

Introduction

This article is the first of a series of reports on culture experiments of mistletoes. The work was begun in September 1911, and will be continued indefinitely. The aim of these experiments is to determine the validity of the several species as now distinguished, their affinities to each other, hosts on which they may be of economic importance or on which they may occasionally occur, and influence of host and condition of host as governed by its environment on the form, color, or other diagnostic characters commonly employed in the classification of these parasites. Since the systematic position and host relationships of several of these plants are not definitely defined, and since they are of great economic importance in many forest regions, it is believed the work will be of considerable value. The plan of these reports is to record as briefly as possible the results of each series of cultures as completed. The present report includes considerable discussion, owing to the necessity of outlining the problems in hand. The detailed discussion of results and technical description of species will be reserved until the conclusion of the experiments.

Methods

For that part of the work being conducted at Missoula, Montana, the opportunities are very favorable. Practically all the species of *Razoumofskya* of any economic importance are of easy access from the laboratory. Members of the field force of the United States Forest Service are aiding in the work by sending in fresh mature specimens of *R. pusilla* on spruce and larch from the Lake states, and of the rare unclassified forms occurring on white and yellow pines in Oregon, Idaho, Utah, and Nevada. A great deal of material of the common forms from all parts of the Northwest has also been contributed. The writer visits regularly the various forests of the Northwest and has made abundant collections of the mistletoes of these regions. The writer is under particular obligations to Professor W. C. WEIR for service in connection with cultures at Bellingham, Washington; to L. H. WEIR for collecting special material; to D. R. BREWSTER of the Forest Service Experiment Station, at Priest River, Idaho, and to J. DUNCAN, Superintendent of Parks of the city of Spokane, for permitting cultures to be made on various exotic conifers; and to E. E. HUBERT of this laboratory for assistance in making cultures.

From 1911 to 1914 inclusive the inoculations were conducted in the open. Seeds were sown on trial hosts of species other than that on which they developed, either in the same vicinity or in widely separate regions. In the latter case trial hosts of the same species as that on which the mistletoe grew were also included. This served to check the viability of the seed, also to bring out differences due to change of environment between the plants resulting from inoculation on the same host species and the plants furnishing the seed. The same was true for the plants on trial hosts other than that on which the parent plant developed. This double procedure demanded copious notes on the conditions of growth and general morphology of the plants furnishing the seed used in inoculations in other regions and the saving of specimens of both sexes for comparison afterward. The same was done with plants resulting from inoculation. In the latter case, where necessary, the infected branch or stem was cut out to prevent the spread of the parasite in new regions. A large number of specimens are

accumulating, but this seemed desirable in case all necessary notes were not taken on both generations. In the case of continuing the inoculations of the same species of mistletoe through several generations on the same host but different individuals, either in the same or different localities, or on different host species, the saving of specimens fully recorded is doubly necessary. This should also furnish some information on the subject of the germinal transmission of characters.

Cultures begun in 1914 are being conducted both in the field and in the greenhouse. This doubles the amount of work, insuring greater dependency on results; and in the case of the indoor work closer study is possible of the life history of a successful inoculation. Indoor work also permits the use of a larger number of trial host species. The seeds germinate more rapidly and results are sooner obtained. One of the chief reasons for maintaining outdoor cultures is to check, whenever possible, under natural conditions, any unusual result obtained in the greenhouse. Cultures in the open have so far proved more successful than those inside, where the same mistletoes and hosts were concerned. If, however, a few unusual hosts are obtained indoors, it must be remembered that it is a new association of host and parasite often not possible in nature; moreover, some of the mistletoes showing the greatest predilection for a particular host or host genus are occasionally found on trees belonging to other genera.

In making the inoculations great care is exercised to attach the seeds at the most vulnerable points, such as in the axils of the leaf sheaths, tender branches, base of terminal buds, and in the denser zone of needles at the nodes. Observations show that infection usually occurs at these places.[1] Before the seeds are transferred to the host they are allowed to stand for a few minutes in water. This causes the mucilaginous coat of the seed to expand. The seeds are then sucked against the point of a dropping pipette and placed firmly in the desired position. After a short time the mucilaginous layer dries, holding the point of the seed in place.

The host material used in the inoculations ranges from seedlings 2 years old to the tender branches of mature forest trees. In case

[1] WEIR, JAMES R., *Wallrothiella Arceuthobii*. Jour. Agric. Research 4:377. 1915.

of the trial host possessing a suberized cortex the seeds are sown only on the first to the sixth year's growth. It has already been demonstrated that infection will not normally take place on older tissues.[2] It has been experimentally proven, however, that by scraping away the dead surface tissues of the bark on parts of branches as old as 7 years, and which still contain chlorophyll, it may be possible to secure infection. The number of seeds sown on each trial host has been maintained at 20 for greenhouse cultures and, owing to possible accident to the seed, 50 for outdoor work. It seemed desirable to try to maintain the seed at a fixed number so that the relative susceptibility of all trial hosts to any one form of mistletoe may be compared. Because it is impossible, especially of cultures in the open, to know that all seeds sown remained on the trees, the relative susceptibility of all trial hosts was further tested in most cases where it was particularly desirable to do so and whenever it was possible, by using a fixed number of trees of any one genus. A record of the source of seed of all trial hosts and of the place where the trees were grown was kept. This seemed desirable in view of the question of influence on the morphology of the parasite. In the case of transplants the trees had not been transplanted very long to the place where cultures were made. The seeds demand a period of rest before germination, and if stored under cool and moist conditions may be carried over and sown in the spring. Sowings as late as April have resulted in successful inoculations. The low temperatures of winter also seem beneficial to the seed, as it is observed that a higher percentage of seeds germinate which have undergone freezing temperatures. This probably accounts for the greater number of positive results obtained in outdoor cultures. If the seeds are stored in warm, dry air, they lose their vitality very rapidly, owing to the evaporation of moisture from the chlorophyllaceous endosperm. Germination tests show that the seeds are capable of germination some 2 weeks before they are normally expelled from the capsule, so that it has been possible to sow the seeds of some species early in September. Care must be taken in sowing seeds on *Larix* before

[2] WEIR, J. R., Mistletoe injury to conifers in the Northwest. U.S. Dept. Agric. Bull. 360. p. 8. 1916.

the leaves have fallen; otherwise the seeds placed on the foliar spurs will be carried away with the falling leaves.

The cultures of the false mistletoes may be considered difficult. There must first be considerable knowledge of the requirements for seed germination, and of the plants afterward, in the case of the work done indoors. Much that is necessary has been learned, and the work is now going on more rapidly. The following is the first detailed report of the culture of mistletoes in this country. Some work of this kind but in another connection has already been reported by the writer (*loc. cit.*).

FIG. 1.—*Razoumofskya campylopoda* on *Pinus ponderosa:* slender, branching form with stems more or less cylindrical at base, pistillate; Oregon coast; reduced one-fourth.

FIG. 2.—*R. campylopoda* on *Pinus ponderosa:* short, thick form with angular stems, staminate and pistillate plants; Oregon coast.

Cultures with yellow pine mistletoes

Razoumofskya campylopoda (Engelm.) Piper and *R. cryptopoda* (Engelm.) Coville, the largest and most conspicuous members of the genus in the United States, are supposedly 2 distinct species occurring on yellow pines. The former (figs. 1, 2, 6) is based on specimens from north Idaho or northeastern Washington, and is principally confined to the coast and northern Rocky

FIG. 3.—*R. cryptopoda* on *Pinus ponderosa:* pistillate; New Mexico; reduced one-fourth. —Photograph by G. G. HEDGCOCK.

Mountain regions.[3] The latter (figs. 3–5), based on specimens from New Mexico, is apparently limited to the southern Rocky Mountain regions. Both plants were originally described from specimens on *Pinus ponderosa*, which is their most common host. A large collection of these plants on *P. ponderosa* and a number of other hosts from their respective regions shows so few constant distinguishing characters by which the plants from the two geographical regions may readily be separated that it seemed desirable to test them out by cultures. Color, branching, thickness of stems, parting of flowers, and position of anthers on the calyx lobes, characters usually employed to distinguish one species from the other, are not always constant in these plants from the several regions in which they are supposed to occur, but apparently merge into one form or the other with change of habitat, just as is the case in any other species having a wide distribution and range of hosts. In a series of experiments recently completed by the writer and not otherwise mentioned in this paper it has

FIG. 4.—*R. cryptopoda* on *Pinus ponderosa:* staminate and pistillate; southern Utah.

[3] PIPER, CHARLES V., Contrib. U.S. Nat. Herb. 11:222. 1906.

been demonstrated that size and color of flowers, stem, fruit, form and division of calyx lobes, slenderness and length of plant, compactness of individual colonies of the northern form depend upon age of the plants and of the infection, nourishment, condition, location, and species of host. In view of these results it seems desirable that the diagnostic characters as now employed in the separation of the large plants on yellow pines should be substantiated by a large number of cultures before they can be held specifically distinct. Experiments involving the transfer of seeds of the northern and coast plant from its various hosts to Rocky Mountain yellow pines, and vice versa, in their respective regions should be of some value in determining the validity of the two alleged species.

FIG. 5.—*R. cryptopoda* on *Pinus chihuahuana:* pistillate; reduced one-half.—Photograph by G. G. HEDGCOCK.

FIG. 6.—*R. campylopoda* on *Pinus ponderosa* as it often appears growing from an advancing cortical stroma in branches of witches' brooms: plants pistillate, mature.

R. occidentalis abietina Engelm. (figs. 7, 8) is a large form of mistletoe found on *Abies* throughout California, Washington, Oregon, and Idaho. It closely resembles the large mistletoes on yellow pines and is described as a variety of the form *R. campylopoda* (figs. 1, 2, 6) (*Arceuthobium occidentale*). The plant is not so large as the latter, but both have the same color variations and bloom and fruit in the same period. The facts that it is usually found in

the same regions where the yellow pine mistletoe occurs, has the same diseases attacking it, and is not found in regions where the typical *R. tsugensis* is most abundant and which it also slightly resembles, indicate that it may be a biological form of the former. The results of a number of cultures involving the three plants mentioned are presented in table I.

FIG. 7.—*R. occidentalis abietina* on *Abies concolor:* staminate and pistillate plants; Oregon.

It will be seen from table I that an effort has been made to sow the seed of the large mistletoes on *Pinus ponderosa* (figs. 1–6) from several localities on as many different hosts as possible and on the same host in widely separate regions. The object of the latter was to try to determine the relationship of the common mistletoes with thick, robust stems on yellow pine in the Rocky Mountain region to the more slender form on the same host in the Pacific Coast region. This problem has been sufficiently outlined previously. The cultures so far do not furnish any evidence that the two forms should be considered identical. Plants in the Pacific Coast region resulting from seed collected in the northern Rocky Mountain region, and vice versa, exhibit various color varia-

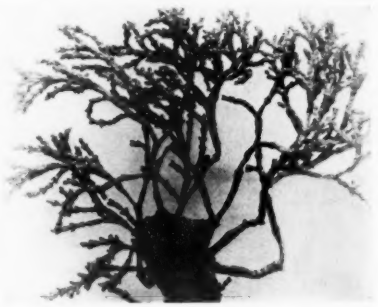


FIG. 8.—*R. occidentalis abietina* on *Abies nobilis:* staminate plants; Oregon.

tions, depending upon the region where grown. The same variation is noted in the robust form when grown in the North. This shows that these plants from the different localities cannot be held specifically distinct on a basis of color. Although color has been one of the chief distinctions between the two, the cultures show that there is no marked difference in the general morphology of each form when grown outside of its original place of collection. True, there are some differences to be noted with respect to size, but it is purely a matter of age of infection. Even after the first maturity these plants, which have a comparatively long life, grow larger by developing additional branches and increasing the thickness of the stem. The comparisons made in the table are based on plants differing widely in age; consequently measurements must vary slightly. Excepting color changes, which were to be expected from varying habitats, the general morphology of the younger plants of the parent colonies were in no particular different from those of the cultures. The cultures have also demonstrated the fact that *R. campylopoda* will infect *Abies*, with considerable variation in color and size of the resultant plants, but closely resembling the form known as *R. occidentalis abietina*. It is interesting to note in this connection that COVILLE[4] refers the plant found on *Abies magnifica* and *A. concolor* directly to *R. campylopoda* (*R. occidentalis* [Engelm.] Coville) with the statement that it is probably the plant that ENGELMANN[5] had previously described under this name (*Arceuthobium campylopodum*). It is further shown that *R. campylopoda* will infect *Picea* and *Larix*, but with difficulty.

This mistletoe also will apparently readily infect *Pinus contorta*, a result repeatedly confirmed in the field. This tree, however, is not a common host. As will be shown in the case of *R. americana*, it is believed that this parasite may be expected to occur on any hard or yellow pine, but with predilection for certain species. The mere assumption that hosts are the determining factors of a species is here shown to be untenable. When a parasitic species will infect hosts from widely separate regions and even genera, and the resulting plants have certain characters varying from those exhibited

[4] Contrib. U.S. Nat. Herb. **4**:192. 1893.

[5] GRAY, ASA, Pl. Lindh. **2**:214. 1850.

TABLE I

SEED COLLECTED SEPTEMBER 25, 1911, ST. MARIES, IDAHO; SEPTEMBER 29, 1912, SANTA BARBARA NATIONAL FOREST, CALIFORNIA; SEPTEMBER 30, 1912, AND AT INTERVALS FROM SEPTEMBER 4 TO NOVEMBER 14, 1914, AT SPOKANE, WASHINGTON, AND IN UTAH, FROM THE LARGE MISTLETOES ON *Pinus ponderosa* COMMONLY KNOWN IN THEIR RESPECTIVE REGIONS AS *R. campylopoda* AND *R. cryptopoda;* CULTURES FROM SEED COLLECTED IN THE SANTA BARBARA NATIONAL FOREST, CALIFORNIA, MARKED *A;* CULTURES FROM SEED COLLECTED AT ST. MARIES, IDAHO, MARKED *B;* CULTURES FROM SEED COLLECTED IN UTAH MARKED *C*.

TRIAL HOSTS			LOCALITY WHERE CULTURES WERE MADE AND DATE	RESULTS OF CULTURES	
Name and number used in cultures	Source of seed	Locality grown		Date of first observation of germination	Results (measurements averaged)
Pinus ponderosa (2).....	Local	Local	Priest River, Idaho 10–6–12, *A**	6–26–13	October 14, 1916: 1 infection on each host; all staminate (*a*) 3 cm. high, deep olive,‡ mature, flowering spikes 1 cm. long (no. 637); (*b*) 3 cm. high; general color, yellowish olive; nodes vinaceous brown (no. 638); parent, 6 cm. high; much branched, yellowish olive (no. 636).
Pinus ponderosa (2).....	Local	Local	Priest River, Idaho 10–4–12, *C**	5–10–13	October 15, 1916: 1 infection on each host, staminate and pistillate; former 3.5 cm. high, robust, thick at base; branches, somewhat paniculate, dark greenish olive; floral spikes 1 cm. long; flowers mostly 3-parted, compressed; latter 5 cm. high, stems thick at base, light brownish olive, mature; seed greenish olive, 3.02 by 1.44 mm.; parent, pistillate; stems light olive yellow, thick at base, robust throughout, 4 cm. high; fruit, olive yellow; seed, 3.04 by 1.45 mm.; staminate, olive yellow; branches, short, 4 cm. high; floral spikes, 1.5 cm. long; flowers, 3 and 4-parted.

Pinus ponderosa (2).....	Cal.	Cal. and Wash.	Bellingham, Wash. 10–10–11,* *B*	4–10–12	September 19, 1915: 3 infections: (*a*) staminate, (*b*) staminate, (*c*) pistillate; all 5 cm. high. (*a*) Stems seal brown; floral spikes, light yellowish olive mature; 1 cm. long; flowers mostly 3-parted; exposed (no. 671). (*b*) Stems uniformly olive yellow; floral spikes 7 mm. long; flowers 3 and 4-parted; developed in shade (no. 672). (*c*) Olive yellow; fruiting spike darker; fruit vinaceous gray; seed 3.02 by 1.45 mm. (no. 673). Parent, pistillate, 6 cm. high; stems tawny olive; fruit pale green-blue gray; seed 3.0 by 1.3 mm. (no. 674); staminate§ 9 cm. high, dark greenish olive; floral spikes 2 cm. long; mostly 4-parted (no 675).
Pinus ponderosa (2).....	Local	Local	Missoula, Mont. 10–25–12*	5–10–13	October 1, 1916: 1 infection; pistillate, 6 cm. high, branched, uniformly light brownish olive; fruit not produced (no. 639); parent 5 cm. high, uniformly dark livid brown (no. 640).
Pinus ponderosa (var. scopulorum) (2).....	S.D.	Mont.	Priest River, Idaho, (Experiment Station) 10–4–12,* *A*	6–19–13	October 15, 1916: 1 infection; pistillate, 2 cm. high, light yellowish olive (no. 641); parent yellowish olive (no. 635).
Pinus ponderosa (2).....	Colo.	Colo.	Priest River, Idaho (Experiment Station) 10–4–12,* *A*	6–19–13	October 15, 1916: 1 infection on each host, staminate and pistillate; former 3 cm. high, light olive, yellow floral spikes 1.5 cm., flowers mostly 3-parted (no. 632); latter 2.5 cm. high, yellowish olive, mature, seed 3.02 by 1.42 mm. (no. 631); parent pistillate; stems isabel, fruit russian blue, seed 3.1 by 1.53 mm. (no. 633); staminate, light brownish olive, much branched, 7 cm. high, floral spikes 2 cm. long, flowers 3 and 4-parted (no. 634).

* Cultures in field. ‡ All color names taken from Ridgway's color standards and nomenclature. § Nearest to pistillate plants from which seeds were used.

TABLE I—*Continued*

Trial hosts			Locality where cultures were made and date	Results of cultures	
Name and number used in cultures	Source of seed	Locality grown		Date of first observation of germination	Results (measurements averaged)
Pinus ponderosa (2).....	Cal.	Ore.	Priest River, Idaho (Experiment Station) 10–4–12*	6–19–13	October 15, 1916: 1 infection, pistillate, 2 cm. high, ecru-olive (no. 642); parent olive yellow, fruit russian blue (no. 643).
Pinus ponderosa (2).....	Local	Local	Priest River, Idaho 10–6–12,* *A*	6–26–13	October 14, 1916: 1 infection, pistillate, 3 cm. high, buffy olive (no. 644); parent 8 cm. high, clay color (no. 645).
Pinus ponderosa (2).....	N.M.	Mont.	Missoula, Mont. 10–10–14*	4–28–15	November 1, 1916: 1 infection, plants appearing; tree died on being transplanted into greenhouse.
Pinus contorta (2).....	Local	Local	Priest River, Idaho 10–6–12,* *A*	6–26–13	October 14, 1916: 1 infection, staminate, 4 cm. high, dark greenish olive, floral spikes 1.5 cm. long, flowers 3 and 4-parted (no. 647); parent pistillate, 7 cm. high, olive-ocher, fruit yellowish olive (no. 648); staminate, 5 cm. high, honey yellow flowered, spikes 1 cm. long, flowers mostly 3-parted.
Pinus contorta (2).....	Local	Local	Priest River, Idaho 10–6–12*	6–26–12	October 14, 1916: 2 infections on 2 different trees; plants pistillate, one 3.5 cm. high, purplish lilac at nodes, remainder of stem reed yellow, fruit not produced (no. 629); the other (no. 630) 2 cm. high, yellowish olive, fruit not produced; parent honey yellow, on same branch with deep yellow staminate plants (no. 628).
Pinus Jeffreyi (2).....	Cal.	Cal.	Missoula, Mont. 12–22–14†	2–16–15	January 3, 1917: 2 infections; plants staminate, 3 cm. high, olive yellow (no. 646); parent yellowish olive (no. 649).
Pinus resinosa (2).....	Minn.	Idaho	Priest River, Idaho (Experiment Station) 10–4–12,* *A*	6–19–13	October 15, 1916: 1 infection, staminate, infected twig stunted, almost dead; one plant attached, remainder fallen, army brown (no. 650); parent light yellowish olive, fruit dark greenish olive (no. 651).

Pinus sylvestris (2).....	Europe	Spokane	Spokane, Wash. 11–10–11*	.....	December 14, 1916: 3 infections, 2 on one stem 2 cm. apart, 1 staminate, 1 pistillate; former 4 cm. high, stems vinaceous drab, floral spikes wax yellow, mature (no. 623); latter 2.5 cm. high, light yellowish olive (no. 623); third infection plant, pistillate, 5 cm. high, not uniformly yellowish olive, internodes purplish gray, fruit not produced (no. 624); parent uniformly yellowish olive, 4 cm. high (no. 625).
Pinus montana (2).....	Europe	Spokane	Spokane, Wash. 11–10–11*	.....	December 14, 1916: 1 on each host, both pistillate, 1 stunted, 4 mm. high, olive yellow (no. 626); other 3.5 cm. high, vigorous, light yellowish olive (no. 626); parent pistillate, 6 cm. high, honey yellow, fruit light drab; staminate stems on same branch, dark greenish olive, floral spikes yellowish olive (no. 627).
Larix occidentalis (4).....	Idaho	Idaho	Priest River, Idaho 10–6–12*	6–26–13	October 14, 1916: 1 infection, plants staminate, 3 cm. high, greenish yellow, had flowered, parent staminate, plain yellow (no. 684).
Larix occidentalis (4).....	Mont.	Mont.	Missoula, Mont. 12–22–14†	2–16–15	May 1, 1916: Penetration of sinker; no further result.
Larix europea (4).....	Europe	Idaho	Missoula, Mont. 10–14–14†	2–16–15	May 1, 1916: Penetration of sinker; no further result
Picea excelsea (1).....	Europe	Idaho	Missoula, Mont. 10–21–14†	2–26–13	November 1, 1916: 1 infection; pronounced fusiform swelling of branch, plants small, barely protruding, 1 mm. in diameter.
Abies grandis (2).....	Local	Local	Priest River, Idaho 10–6–12*	6–26–13	October 14, 1916: 1 infection, pistillate plants 3 cm. high, honey yellow (no. 652); parent (no. 643).
Abies concolor (2).....	Ore.	Cal.	Spokane, Wash. 11–10–11,* *B*	.....	October 13, 1916: 2 infections; staminate plants 4 cm. high, mature, dark greenish olive (no. 653); pistillate, 3 cm. high, dark greenish olive (no. 654); parent (no. 643).

*Cultures in field. †Greenhouse. ‡All color names taken from Ridgway's color standards and nomenclature.

by the parent when growing on what we may term the mother host, the limitations of a species are naturally more difficult to define. Notwithstanding this change, however, a good species should be sufficiently characteristic on any host and under any of the ordinary conditions of growth as to be readily recognized by one having a wide knowledge of the plant in the field. We do not think of the low, scrubby Douglas fir of central Montana as anything different from the gigantic form of this tree occurring in the Puget Sound region.

It is of considerable economic importance that *R. campylopoda* will infect *Pinus resinosa*, *P. sylvestris*, and *P. montana*, and may be expected to be a serious pest on these trees in localities where conditions are favorable. In drier sites of the Lake states and, in fact, throughout the Northeast, where it is proposed to plant *P. resinosa*, this mistletoe would undoubtedly grow luxuriantly, and care should be exercised against its introduction into these regions on nursery stock during the early period of infection.

Seeds of *R. campylopoda* were sown on the following pines in most cases in the greenhouse, but either due to the poor quality of the seed, loss of seed, or low vigor of the trial hosts the results were mostly negative. This does not mean, however, that all of the species mentioned here are immune. In a few cases infection did occur on species not mentioned in the table, but the results were of a nature that it is thought best not to report them at this time. These were *Pinus Banksiana*, *P. mayriana*, *P. Strobus*, *P. Cembra*, *P. cembroides*, *P. edulis*, *P. Lambertiana*, and *P. monticola*. Sowings made on *Pseudotsuga taxifolia*, *Larix leptolepis*, *Tsuga heterophylla*, *Thuja plicata*, *T. occidentalis*, *Cupressus arizonica*, *Picea Engelmanni*, *P. canadensis*, *Populus tremuloides*, *P. trichocarpa*, *Betula occidentalis*, *Alnus tenuifolia*, *Acer glabrum*, and *Prunus demissa* resulted negatively.

SUMMARY.—Results of cultures so far indicate that the mistletoes known under the names *Razoumofskya campylopoda* and *R. cryptopoda* are distinct. Each form, however, may exhibit considerable variation, due to geographic location and host. The relationship of the two forms will be further considered when a number of experiments now being conducted are completed.

Cultures show that the plant known as *R. occidentalis abietina* on *Abies* is in all probability a biological form of *R. campylopoda*. The taxonomic position of the plant, however, cannot be established with any certainty until it is successfully grown on yellow pine.

FIG. 9.—*R. laricis* on *Larix occidentalis:* staminate and pistillate plants.

Cultures with larch mistletoe

From the fact that this parasite, *R. laricis* Piper (figs. 9, 10), exhibits considerable variation under different conditions of growth and will occasionally grow on other hosts than *Larix*, it seemed desirable to study the species in culture. The chief results of these experiments are embodied in table II. These results indicate that *Larix* is the true host genus for *R. laricis*. The fact that 6 trees of *Larix occidentalis* were infected out of 6 on which seed were sown demonstrated the close affinity of the host and parasite. The readiness with which *R. laricis* infects *Larix europea* and *L. leptolepis*, the common Japanese larch, shows that this parasite may be expected to cause serious injury to plantations of these species not only in America, but in many parts of Europe and Japan as well, wherever climatic conditions are

FIG. 10.—*R. laricis* on *Larix occidentalis:* pistillate plants; reduced one-half.

TABLE II

SEED COLLECTED AT ST. MARIES, IDAHO, SEPTEMBER 24, 1911; PRIEST RIVER, IDAHO, SEPTEMBER 20 AND 30, 1912; FROM *R. laricis* ON *Larix occidentalis*; ALL CULTURES MADE IN THE FIELD.

TRIAL HOSTS			LOCALITY WHERE CULTURES WERE MADE AND DATE	RESULTS OF CULTURES	
Name and number used in cultures	Source of seed	Locality grown		Date of first observation of germination	Results (measurements averaged)
Larix occidentalis (2).....	Local	Local	Coeur d'Alene, Idaho 11-5-11	.....	August 25, 1915: 2 infections; plants staminate, 2.5 cm. high; flowers reed yellow, 3 and 4-parted, stems olive yellow; parent lime green, upper half of fruit purplish lilac.
Larix occidentalis (2).....	Local	Local	Blue Lake, Idaho 10-3-12	6-18-13	October 14, 1916: 2 infections on one host; plants pistillate, 4 cm. high, stems dark livid purple; spikes olive yellow developed in direct light; parent uniformly light yellowish olive, grew in diffused light.
Larix occidentalis (2).....	Local	Local	Missoula, Mont. 10-30-12	6-6-13	September 8, 1915: 2 infections, pistillate, 4.5 cm. high, stems olive ocher, fruit lime green, culture in dense shade; parent dark olive buff, fruit with pale bluish lavender points, growing in strong light.
Larix europea (2).....	Europe	Wash.	Spokane, Wash. 11-11-11	.....	October 13, 1916: 3 infections, 1 staminate, 2 pistillate; former 3 cm. high, dark vinaceous brown, mature in 1914, flowers lime green, flowers mostly 3-parted (no. 658); latter 2 cm. high, joints dark vinaceous brown, fruit dark greenish olive (nos. 656 and 657); parent; pistillate stems dark livid brown, fruit light yellowish olive (no. 659); staminate stems on same branch, olive yellow flowers, mostly 4-parted (no. 685).

Larix leptolepis (2).....	Japan	Idaho	Priest River, Idaho 10–4–12	6–19–13	October 15, 1916: 1 infection, pistillate, 2 cm. high, stems light yellowish olive, fruit with bluish tips (no. 654); parent dark livid purple (no. 655)
Pinus ponderosa (6).....	Local	Local	Coeur d'Alene, Idaho 11–7–11	.....	August 25, 1915: 1 infection, plants pistillate, 4 cm. high, dark greenish olive, vigorous at time of collection (no. 662); parent honey yellow (no. 663).
Pinus contorta (6).....	Local	Local	Blue Lake, Idaho 10–6–12	6–18–13	October 14, 1916: 1 infection, staminate, 3 cm. high, stems yellowish olive, joints dark vinaceous drab, flowers distantly alternate-opposite, mostly 3-parted (no. 660); parent; pistillate, stems dark vinaceous brown, fruit light yellowish olive (no. 661); staminate, stems on same host anthracene purple, flowers mostly 4-parted, closely alternate-opposite.
Abies grandis (6).....	Local	Local	Blue Lake, Idaho 10–2–12	6–18–13	October 14, 1916: 1 infection; staminate, apparently vigorous for a time, but at this date plants were dead and mostly fallen; original color not determined with certainty but appeared purplish (no. 664); parents light yellowish olive with darker colored fruit (no. 665).

favorable. The nature of the results on *Pinus ponderosa* and *P. contorta*, although demonstrating that this mistletoe under very favorable conditions will infect yellow pines, does not show any great affinity for the genus. When it is recalled that 900 seeds were sown on 18 individuals of *Pinus ponderosa*, each receiving 50 seeds, resulting in one infection, and one infection on *P. contorta* out of 12 trees tested with 600 seeds, the relationship between these 2 tree species and the larch mistletoe cannot be very close. The same is apparently true with regard to the infection of *Abies grandis*. Six trees were tested with the usual number of seeds, but only 1 infection resulted, which later died. These cultures also show that seeds germinating in the most vulnerable places only cause infection. Out of 500 seeds sown on *Larix* only 10 were able to cause infection, although apparently all the seeds which remained on the trees germinated. All were sown on parts of branches or shoots not over 6 years old, and care was taken to place the seeds favorably. It is to be expected that some of the seeds in outdoor cultures are removed by wind, rain, snow, insects, or birds. The observations relative to the favorableness of seed placement do not apply in the same way to the cultures on *Pinus* and *Abies*, since the larch mistletoe does not exhibit any marked affinity for these genera. That the same species of mistletoe growing on different hosts or under different conditions on the same host may exhibit different morphological characters is clearly demonstrated by these cultures.

Since these experiments with the larch mistletoe were started, the following field observations have been made near Fernan Lake, Idaho. A large veteran western larch severely infected with *R. laricis* was left standing in a clearing which reseeded to *Pinus ponderosa* and *P. contorta*. From one each of these species growing directly under the larch typical, although small, specimens of the larch mistletoe bearing both pistillate and staminate plants were collected. The only true pine mistletoe in the immediate vicinity was *R. americana*. In a canyon near Missoula, Montana, where the larch is seriously infected with *R. laricis* and the pine mistletoes are not known to occur, specimens of the former have been collected from a single infection on *Pinus contorta*. These results are very

much at variance with previous ideas of the host affinities of *R. laricis*, but they should not alter in the least the economic situation, since infections very rarely occur. The fact that both pines and larches are resinous may explain the occasional occurrence of the parasite on the former hosts. Although great pains were taken to place the seed in favorable places on the trial hosts, the results on the following species were negative: *Pseudotsuga taxifolia*, *Pinus monticola*, *Picea Engelmanni*, *Thuja plicata*, *Tsuga heterophylla*, *Taxus brevifolia*, *Juniperus communis*, *Populus tremuloides*, *P. trichocarpa*, *Betula occidentalis*, *Alnus tenuifolia*, and *Salix Bebbiana*. Field observations on the intermingling of the branches of most of these species with severely infected branches of larch-bearing pistillate plants confirm the results of the cultures. Such observations, however, cannot be used as conclusive evidence for determining the host range for any one species of mistletoe.

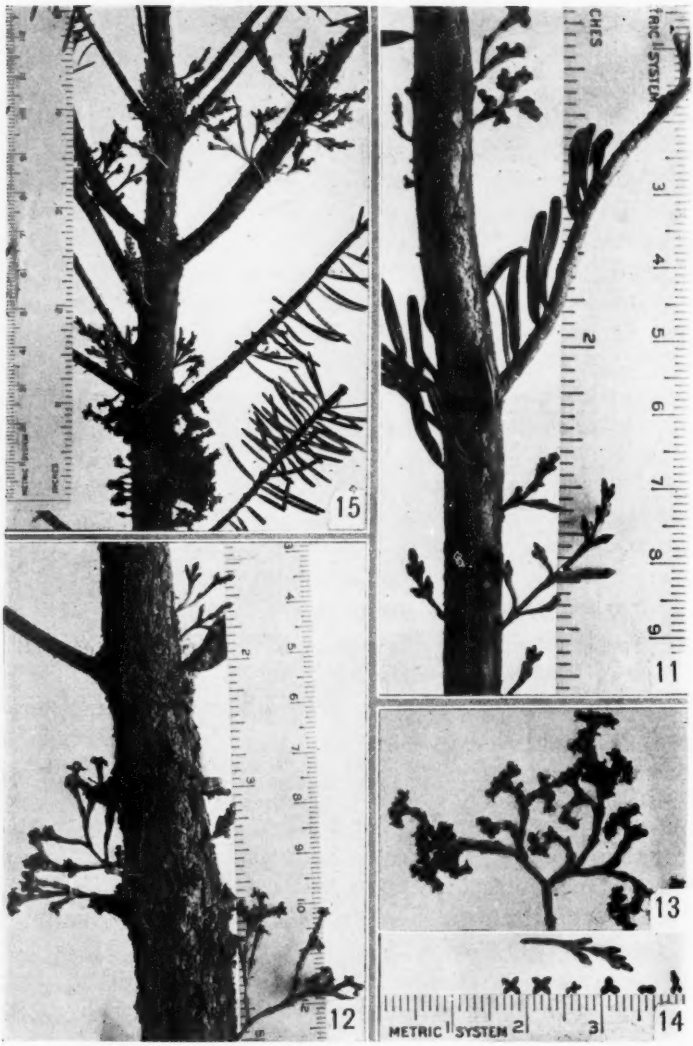
SUMMARY.—The hosts of *Razoumofskya laricis* are *Larix occidentalis*, *L. Lyalli*, *L. europea*, *L. leptolepis*, *Abies grandis*, *Pinus ponderosa*, and *P. contorta*. The parasite is known to be of economic importance to the first named species only. The plant resulting from an infection on any other host than that on which it normally grows exhibits considerable change in morphology and also in vigor. That different degrees of exposure with respect to light very greatly influence the color of the plants is very clearly demonstrated.

Cultures with Razoumofskya species having purple flowers

A group of small mistletoes found in the western United States has one character in common with *R. pusilla* of the East, namely, deep purple flowers.[6] They are *R. Douglasii abietina* (Engelm.) Piper[7] on *Abies* (figs. 11, 12), *R. Douglasii* (Engelm.) Kuntze on *Pseudotsuga* (fig. 15), and a small form on *Picea* (figs. 13, 14). A careful comparison of representative collections of these 3 plants from varied environments shows no constant characters by which they may be held as distinct species. All three have 2, 3, or rarely

[6] WEIR, J. R., *Wallrothiella Arceuthobii*. Jour. Agric. Research 4:372. 1915.

[7] Reported by ENGELMANN under the name *Arceuthobium Douglasii* var. *abietinum* in S. Watson, Bot. Cal. 2:106. 1880.



FIGS. 11–15.—Fig. 11, *R. Douglasii* on *Abies grandis*, staminate and pistillate plants; fig. 12, *R. Douglasii* on *Abies lasiocarpa*, staminate and pistillate plants; fig. 13, small purple-flowered form on *Picea Engelmanni*, staminate plant, natural size; fig. 14, small purple-flowered form on *Picea Engelmanni*, staminate flowers and pistillate plant; fig. 15, *R. Douglasii* on *Pseudotsuga taxifolia*, staminate and pistillate plants.

4-parted purple flowers, solitary or clustered, simple or branched, according to age of infection, bloom and fruit in the same season; and size of fruit, flower, plant, and color of stems show some variations under different conditions of growth. Cross inoculations involving these forms should demonstrate whether or not all 3 are identical with *R. Douglasii*. The results of a series of cultures are given in tables III and IV.

At the time these cultures with *R. Douglasii* and *R. Douglasii abietina* were made seeds of the form on *Picea* were not available. The plant is not morphologically different from the other two, and cultures now under way indicate that it will infect *Abies* and *Pseudotsuga*. The evidence so far obtained is so pointedly in favor of the view that all 3 forms are identical that there can be little room for doubt. We find, for instance, that *R. Douglasii* will infect *Abies grandis*, *A. lasiocarpa*, and *A. concolor*, which are hosts for *R. Douglasii abietina*. No marked morphological differences are found in the resultant plants and their parents, any more than is to be expected from a change of host or condition of growth. The same is true for the culture of this mistletoe on *Picea Engelmanni*. The evidence that all 3 forms are identical is further strengthened by the fact that *R. Douglasii abietina* from *Abies lasiocarpa* will infect *Pseudotsuga taxifolia* and *Abies grandis*, and that it is possible to fertilize the pistillate flowers of this form on the latter host with pollen from plants on *Pseudotsuga*. These results demonstrate the relationship of the 3 small purple-flowered forms here considered. The two forms on *Abies* and *Picea* should be considered identical with *R. Douglasii* in view of the foregoing results. It has already been pointed out that, in the writer's experience, the plants on *Abies* and *Picea* are in most cases found in localities where *R. Douglasii* abounds. If the former were specifically distinct, with inherent tendencies to select their particular hosts, they should in the light of our knowledge of the well defined species be more abundant. On the contrary, they are never found in any quantity. The conclusion that *R. Douglasii* does not abundantly infect other trees than Douglas fir is also shown by the following observations. The writer has looked several times in vain for infection of this species on *Abies* and *Picea* when the

TABLE III

SEED COLLECTED OCTOBER 28, 1911, SEPTEMBER 20, 1912, AND OCTOBER 1, 1914, AT MISSOULA, MONTANA, FROM *R. Douglasii* ON *Pseudotsuga taxifolia*.

TRIAL HOSTS			LOCALITY WHERE TESTS WERE MADE AND DATE	RESULTS OF CULTURES	
Name and number used in cultures	Source of seed	Locality grown		Date of first observation of germination	Results (measurements averaged)
Pseudotsuga taxifolia (2)	.....	.....	Priest River, Idaho 10–5–12*	6–17–13	April 14, 1916: 1 infection; staminate, 1.5 cm. high, solitary, dark olive gray, flowers blackish purple; parent, light yellowish olive with lighter colored flowers; former in direct light, latter in shade.
Abies lasiocarpa (2)	Mont.	Mont.	Missoula, Mont. 10–24–14†	2–8–15	Pronounced swelling at several points; apparent infections; plants never appeared.
Abies grandis (2)	Mont.	Mont.	Missoula, Mont. 10–24–14†	2–6–15	One infection but died out; host in poor condition.
Abies grandis (2)	.....	.....	Priest River, Idaho 10–5–12*	6–17–13	October 14, 1916: 1 infection; pistillate, plants 2 cm. high, simple, solitary, light yellowish olive, fruit dark greenish olive, mature, flowers fertilized from plants resulting from inoculation on *Pseudotsuga* which were 2 m. distant, seed were immediately sown on *Pseudotsuga;* parent brownish, 3 cm. high, were deep olive gray with slightly lighter colored fruit.
Abies concolor (2)	.....	.....	Spokane, Wash. 11–10–11*	.....	April 14, 1916: 1 infection, staminate, 2 cm. high, solitary, slender, light yellowish olive, flowers vernonia purple, 2 and 3-parted, in shade (no. 670); parent, 3 cm. high, clustered, branched, yellowish olive, with purplish fruit, grew in direct light; staminate, 2.5 cm. high, clustered, branched, rather thick for species, olive buff, flowers vernonia purple, 3-parted, grew in shade.
Picea Engelmanni (2)	.....	.....	Priest River, Idaho 10–5–12*	6–17–13	April 14, 1916: 1 infection; staminate, 2.5 cm. high, solitary, branched, flowers vernonia purple, plants light yellowish olive (no. 676); parent, pistillate, 3 cm. high, simple, solitary, dark olive gray with purplish tipped fruits (no. 677); staminate, 3 cm. high, clustered, simple, flowers dark corinthian purple.

TABLE IV

SEED COLLECTED ON BALD MOUNTAIN NEAR LAKE PEND OREILLE OCTOBER 2, 1912, FROM *R. Douglasii abietina* ON *Abies lasiocarpa*.

TRIAL HOSTS			LOCALITY AND DATE	RESULTS OF CULTURES	
Name and number used in cultures	Source of seed	Locality grown		Date of first observation of germination	Results (measurements averaged)
Pseudotsuga taxifolia (4)..	Local	Local	Priest River, Idaho 10–6–12	6–17–13	April 14, 1916: 1 infection; staminate, 2 cm. high, solitary, branched, light yellowish olive, flowers blackish purple (no. 678); parent (no. 679), not differing in any particular feature with the exception of staminate flowers of parent plants were slightly larger and of a lighter color.
Pseudotsuga taxifolia (4)..	Local	Local	Blue Lake, Idaho 10–9–12	6–18–13	April 14, 1916: 1 apparent infection, swelling of branch; plant did not appear.
Abies grandis (4).....	Local	Local	Priest River, Idaho 10–8–12	6–17–13	October 14, 1916: 1 infection; pistillate, solitary, branched, 3 cm. high, light yellowish olive, fruit mature, artificial pollination from no. 678, brownish purple at tips, seed immediately sown on *Pseudotsuga;* parent, 3.5 cm. high, simple, solitary, dark olive-gray, fruit dark greenish olive (no. 681).

latter grew in absolute contact with brooms on Douglas fir bearing pistillate plants. As previously stated, however, this is not conclusive evidence of the host range of a species. Accident of infection is too great; besides, trees growing in such juxtaposition are very often suppressed, thus reducing the amount of vulnerable tissue. These results were obtained only by the most careful placing of the seeds at the most susceptible points. In the course of years such conditions occur in nature. Afterward, as a matter of course, further infection from the parent tree to others of its kind may be easier. The type of broom produced by *R. Douglasii* varies with age and host. On hosts with strongly excurrent growth, such as *Abies lasiocarpa* and *A. grandis*, the brooms are usually erect, but drooping or swaying forms occur. The erect type of broom is common on *Pseudotsuga taxifolia* during the first years of infection, but later may assume the weeping willow form.

Seeds from plants on *Abies lasiocarpa* were sown on a single individual each of *Tsuga heterophylla*, *Larix occidentalis*, *Pinus monticola*, *Thuja plicata*, and *Populus trichocarpa*, but without results. Seeds from plants on *Pseudotsuga taxifolia* were without result on these hosts and also on *Larix europea*, *Picea sitchensis*, *P. canadensis*, *P. excelsea*, *P. Parryana*, *Sequoia gigantea*, *Pinus ponderosa*, *P. contorta*, *P. Jeffreyi*, *P. sylvestris*, *Betula occidentalis*, *Alnus tenuifolia*, and *Pyrus*. The several species of *Picea* were not in a vigorous condition, having been transplanted only a short time before the seed were sown.

SUMMARY.—The foregoing cultures indicate that *Razoumofskya Douglasii abietina* is identical with *R. Douglasii*. The hosts of *R. Douglasii* as known to the writer are *Pseudotsuga taxifolia*, *Picea Engelmanni*, *Abies concolor*, *A. grandis*, *A. lasiocarpa*, *A. nobilis*, and *A. amabilis*. The species is of economic importance only on *Pseudotsuga taxifolia*.

Cultures with lodgepole pine mistletoe

This species (*R. americana* [Nutt.] Kuntze) (figs. 16, 17) is one of the most characteristic of the genus. In order to determine its host range, the results of some recent cultures are presented in table V. It is shown that *Pinus contorta* is the true host of *R.*

americana, but that occasionally other hard pines are attacked. The writer has previously reported the occurrence of this mistletoe on *Pinus attenuata*, *P. Jeffreyi*, and *P. ponderosa*, and it has long been known to be common on *Pinus Banksiana* in Canada. The fact that this mistletoe will infect *Pinus montana*, the common mountain pine of Europe, further supports the writer's contention that it may be expected to occur occasionally on any of the hard or yellow pines, and also is a warning that the parasite would probably find a favorable home in Europe. The plant apparently attacks the yellow pines other than *Pinus contorta* with difficulty. Such infections are by no means common, and frequently result in some morphological changes in the plant. These changes, however, may not be any more marked than those the plant may exhibit when developing under various light intensities or varying conditions of nourishment on its regular host. If *R. americana* exhibits a certain antipathy to other yellow pines, it apparently has a much greater aversion to white pine. That the species will infect white pines but with difficulty, and will never be of consequence in this respect, is shown by the discovery of two infections on *Pinus albicaulis* near Darby,

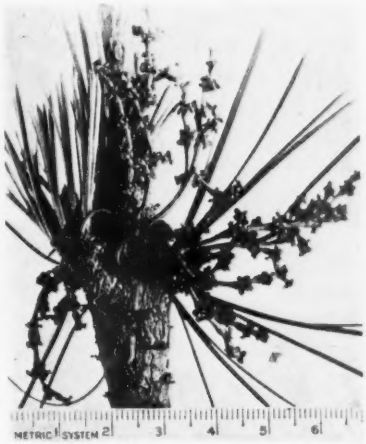


FIG. 16.—*R. americana* on *Pinus ponderosa:* staminate plants.

FIG. 17.—*R. americana* on *Pinus contorta:* pistillate plants.

TABLE V

SEED COLLECTED AT LIBBY, MONTANA, OCTOBER 18, 1911; PRIEST RIVER, IDAHO, OCTOBER 1, 1912; AND AT COEUR D'ALENE, IDAHO, NOVEMBER 14,1914, FROM *R. americana* ON *Pinus contorta*.

TRIAL HOSTS			LOCALITY WHERE CULTURES WERE MADE AND DATE	RESULTS OF CULTURES	
Name and number used in cultures	Source of seed	Locality grown		Date of first observation of germination	Results (measurements averaged)
Pinus contorta (4).....	Local	Local	Blue Lake, Idaho 10–2–12*	6–18–13	April 13 and October 14, 1916: 1 infection on each tree, slight swelling of branch on stem, 2 staminate, 2 pistillate (no. 666), not varying in any marked degree from parent plants.
Pinus ponderosa (4).....	Local	Local	Blue Lake, Idaho 10–2–12*	6–18–13	April 13, 1916: 2 infections at the same node on a single stem, only 1 tree infected, plants staminate and pistillate; former 4 cm. high, stems olive yellow; latter 5 cm. high, stems yellowish olive, fruit light dull glaucous blue (no. 667); parent; staminate, 8 cm. high, light yellowish olive with older parts of stem deep grape green; pistillate, 4 cm. high, uniformly dark olive gray, both stems and fruit (no. 668).
Pinus ponderosa (1).....	Mont.	Mont.	Missoula, Mont. 1–12–15†	2–16–15	October 10, 1915: 1 infection, slight swelling, branch died, plants did not appear.
Pinus montana (dwarf form) (4).....	Europe	Spokane	Spokane, Wash. 11–10–11*	.....	December 14, 1916: 1 infection; staminate, 5 mm. high, pronounced swelling of branch, excessive infiltration of resin, 2 small plants barely appeared in 1915, died out; another plant appeared just above original point of infection but remained small (no. 669).

* Cultures in field. † Greenhouse.

Montana. The parasite in this case caused unusually large and elongated swellings on the main stem of young trees, but the plants apparently were never able to come to maturity, remaining about 5–8 mm. high. One of the pines was transplanted into the greenhouse, and the context of the swelling shriveled up in a manner indicating that it was composed of very spongy tissues. The tree, however, remained living. The fact that 220 seeds of *R. americana* were sown on 6 different species of white pines with no result except the germination of the seeds further supports this observation. The trees tested were *Pinus Lambertiana*, *P. monticola*, *P. Strobus*, *P. edulis*, *P. cembroides*, and *P. Cembra*. *R. americana* is reported by COULTER and NELSON[8] on *Pinus flexilis*. The results of sowings on *Larix europea*, *L. occidentalis*, *Picea sitchensis*, *P. Engelmanni*, *P. excelsea*, *Abies nobilis*, *A. lasiocarpa*, *A. grandis*, *Tsuga heterophylla*, *Pseudotsuga taxifolia*, *Thuja plicata*, *Taxus brevifolia*, *Populus trichocarpa*, *Betula occidentalis*, and *Alnus tenuifolia* were negative.

SUMMARY.—The hosts of *Razoumofskya americana* are *Pinus contorta*, *P. Banksiana*, *P. attenuata*, *P. Jeffreyi*, *P. montana*, *P. ponderosa*, *P. flexilis*, and with difficulty *P. albicaulis*. The plant is of economic importance so far as known only on the two first named species. Morphological changes are induced by change of host or condition of growth, but not to an extent that this, the most characteristic of all members of the genus on pines, could be confused.

Cultures with hemlock mistletoe

In the St. Joe National Forest, Idaho, are several areas of almost pure stands of *Tsuga heterophylla* heavily infected with *R. tsugensis* (figs. 18, 19). In the border zones of these areas a form of mistletoe has been collected on *Abies grandis* and *A. lasiocarpa* which varies in a number of details from the form collected on the same hosts in regions where the large mistletoe on *Pinus ponderosa* occurs. In order to see whether this is a case of *R. tsugensis* infecting other hosts than the common western hemlock, and also to determine its host range in general, the cultures given in table VI were made.

[8] New Manual of Botany of the Rocky Mountains. 146. 1909.

R. tsugensis is not confined to species of *Tsuga* as heretofore believed, but will infect *Abies lasiocarpa*. The mistletoe most closely resembling *R. tsugensis* in point of color and size is the form *R. occidentalis abietina*, but as the results from cultures stand at present there is apparently no relation between them. The foregoing results indicate that the plant occasionally found on firs in the same vicinity with *R. tsugensis* is the common hemlock mistletoe,

FIG. 18.—*R. tsugensis* on *Tsuga heterophylla:* staminate and pistillate plants; large form.

and also that this species may be expected to occur occasionally on other hosts than hemlock. Cultures may be considered fully completed when the plants found on *Abies* in the vicinity of *R. tsugensis*, also the form on *Abies* which has been referred to the yellow pine mistletoe, are shown by culture to infect *Tsuga* and *Pinus* respectively.

SUMMARY.—Seeds were also sown on *Abies grandis*, *Pinus ponderosa*, *Picea orientalis*, *Larix occidentalis*, and *Pseudotsuga taxifolia*, but the results were negative. The hosts of *Razoumofskya*

tsugensis are *Tsuga heterophylla*, *Tsuga canadensis*, and *Abies lasiocarpa*. So far as the present cultures show, the hemlock mistletoe will not infect *Pinus*, *Picea*, *Larix*, and *Pseudotsuga*. The fact that this mistletoe will infect *Tsuga canadensis* indicates the possibility of it becoming a pest in the native regions of other species of hemlock and is a condition to be guarded against.

Conclusion

Cultures at present indicate that *R. campylopoda* and *R. cryptopoda* are not identical. Each form may exhibit considerable variation, due to geographic location and host. It is shown that *R. campylopoda* will infect *Pinus resinosa*, and care must be taken to prevent it from getting a foothold in the eastern United States. It will also infect *Pinus sylvestris* and *P. montana*, and should be prevented from entering Europe or plantations of these trees in America. It is also indicated that the plant known as *R. occidentalis abietina* is a biological form of *R. campylopoda*.

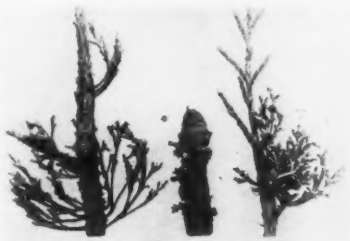


FIG. 19.—*R. tsugensis* on *Tsuga mertensiana:* staminate (center) and pistillate plants; small form; reduced one-half.

R. laricis will infect *Larix europea*, *L. leptolepis*, *Abies grandis*, *Pinus ponderosa*, and *P. contorta*. All are new hosts for this species except the last. The parasite apparently readily infects the Japanese and European larch and would be expected to cause serious damage to these trees. *Abies grandis*, *Pinus contorta*, and *P. ponderosa* are infected with difficulty. This parasite so far as known at present is of economic importance only on *Larix occidentalis*.

The mistletoe known under the name *R. Douglasii abietina* is shown to be identical with *R. Douglasii* and should be written under the latter name. *R. Douglasii* is only of importance on *Pseudotsuga taxifolia*.

TABLE VI

SEED COLLECTED AT MISSOULA, MONTANA, JANUARY 22, 1915, ADHERING TO BROOMS AND MISTLETOE PLANTS ON *Tsuga heterophylla* SENT FROM ASHFORD, WASHINGTON, AND FROM THE SAME HOST IN ST. JOE NATIONAL FOREST, IDAHO, SEPTEMBER 30, 1914

TRIAL HOSTS			LOCALITY WHERE CULTURES WERE MADE AND DATE	RESULTS OF CULTURES	
Name and number used in cultures	Source of seed	Locality grown		Date of first observation of germination	Results (measurements averaged)
Abies lasiocarpa (1).....	Mont.	Mont.	Missoula, Mont. 1–28–15†	2–29–16	January 3, 1917: 1 infection, staminate, 2 cm. high, leaf green (no. 686); parent, 6 cm. high, light yellowish olive.
Tsuga canadensis (1).....	Wis.	Wis.	Spokane, Wash. 10–7–13*	5–1–15	October 12, 1916: 1 infection, staminate, very slight swelling, 4 cm. high, dark greenish olive (no. 682); parent light brownish olive (no. 683).

* Cultures in field. † Greenhouse.

R. americana will infect both hard and soft pines, the latter with difficulty, and is of importance only on *Pinus contorta* and *P. Banksiana* of the former group. This mistletoe will infect *Pinus montana* and may be of consequence if introduced into Europe.

R. tsugensis will infect *Abies lasiocarpa*, thus possibly explaining the position of certain rare plants occasionally found on *Abies* in the vicinity of the hemlock mistletoe. This parasite will infect *Tsuga canadensis* and would probably cause serious damage to this tree in the East.

Cultures show very clearly that many of the characters employed in the classification of the false mistletoes vary with change of host, geographical location, and with various other environmental factors. This indicates that only the broader and plainly evident lines of demarcation should be employed in their classification.

OFFICE OF INVESTIGATION IN FOREST PATHOLOGY
BUREAU OF PLANT INDUSTRY
MISSOULA, MONT.

CHEMICAL CHANGES ACCOMPANYING ABSCISSION IN COLEUS BLUMEI

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 240

HOMER C. SAMPSON

Introduction

VON MOHL (13) in 1860 was the first to announce that previous to the fall of the leaf there is formed near the base of the petiole a definite separation layer in which abscission always occurs by the separation of the cells from each other with their walls still intact. The xylem tubes not being included in this separation layer are finally ruptured mechanically, and the leaf falls. He also called attention to the fact that abscission and the formation of a protective tissue are two very distinct processes, and that the latter process might either precede or follow the former. WIESNER (17) in 1871 confirmed the observation of VON MOHL in the main, and formulated the theory that the dissolution of the intercellular substance of the cells of the separation layer is caused by the action of organic acids developed in the leaves. In 1886 MOLISCH (14) suggested that a gum ferment might be the cause of this dissolution process. Two years later MANGIN (11), upon his discovery of the pectic nature of the middle lamella of cell walls in plants, indirectly advanced the knowledge of abscission. Since this discovery the abscission process has generally been referred to as a dissolution of the pectose and calcium pectate of the middle lamella. LLOYD (8, 9), assuming the organic acid theory of WIESNER, speaks of the process as a hydrolysis of these pectic compounds, and later (11) of cellulose also.

The anatomical workers disagree somewhat on the amount of the cell wall altered during abscission. LEE (7) in 1911 reported the disappearance of the middle lamella only, and two years later HANNIG (6) reported the same condition in the abscission of flowers, with the exception of a species of *Mirabilis* and of *Oxybaphus*, in which the entire cell wall disappeared. On the other hand, TISON

(15) as early as 1900, working with numerous species studied later by LEE, stated that in general the secondary membranes of the cell wall also undergo alteration and disappear, leaving only the thin tertiary membrane lining the cell lumen. LLOYD (9, 10) has recently found the same condition in the abscission of cotton bolls and also in *Mirabilis*, instead of the disappearance of the entire cell wall as reported by HANNIG. It seems improbable that the presence of weak organic acids would be sufficient to account for this extreme alteration of the cellulose walls of these cells. Certain authors have suggested that the catalytic effect of enzymes may be an important factor in abscission, but experimental evidence has been wanting.

Aside from his organic acid theory, WIESNER (21) in 1905 suggested increased turgor as a cause of abscission under conditions of forced leaf-fall. FITTING (5) in 1911 accepts this view to account for the rapid abscission of petals when forced. The suggestion lacks experimental confirmation, and the work of HANNIG (6) indicates less need for its assumption.

The external factors capable of accelerating leaf-fall are extremely diversified. These have been summarized in the main by LLOYD (8). The more important are high and low light intensity, high and low water supply, high temperatures and frost, low concentrations of anesthetics, toxic concentrations of acids and salts, and wounding of the blade. On the other hand, low concentrations of oxygen and high concentrations of anesthetics retard leaf-fall, a state of rigor being produced by the latter.

The internal changes affected by these various external factors have received very little critical study. The work discussed in the present paper was undertaken to determine some of the internal changes accompanying abscission of leaves in *Coleus Blumei* var. Golden Bedder. This plant was chosen for study partly on account of its ease of propagation, but mainly for its simplicity of analysis owing to the absence of protective tissue at the time of abscission.

Anatomy

In order to appreciate fully the chemical changes taking place in the abscission layer, it is necessary not only to compare the

abscission layer with the adjacent regions of the petiole, but also to follow the changes in the abscission layer itself from the time of its formation to the fall of the leaf. This latter process is most easily accomplished in *Coleus* by beginning with the terminal bud and taking the leaves as they appear in order down the stem, from the youngest to the oldest. On this basis the following description is applicable to *Coleus* plants growing in 4-inch pots under greenhouse conditions, with each plant bearing 8 pairs of leaves, and with the eighth pair in the process of abscissing.

In the first 2 pairs of leaves below the terminal bud there is no evidence of an abscission layer. The cells in the region where the abscission layer is later to occur are in the enlargement period of growth. The formation of the abscission layer in *Coleus* is usually initiated in the third pair of leaves below the terminal bud. Cell divisions in 2–4 layers of cells across the base of the petiole of these leaves begin in the epidermal and cortical region and gradually extend inward, reaching the phloem about the time the leaves appear as the fifth pair in order below the terminal bud. In the sixth pair of leaves the formation of the abscission layer is practically completed and involves all the tissue of the petiole except the xylem tubes. Growth of the leaf in general ceases long before this period is reached. In the majority of cases the fourth pair of leaves below the terminal bud are fully expanded. The formation of the abscission layer in *Coleus* therefore begins a short time before the maturity of the leaf and continues for a considerable period afterward. As a rule the layer is 8–12 cells in thickness. These cells always remain smaller than the neighboring cells of the adjacent regions of the petiole and their walls are somewhat thinner. At the time of abscission alteration of the walls of the cells of the abscission layer is quite general, but a continuous plane of separation is finally formed somewhat nearer the distal side of this layer. This extreme alteration of cell walls is localized in the abscission layer and is not found throughout the entire leaf, as was reported by WIESNER (**18**).

While this description is true in general for *Coleus* plants bearing 8 pairs of leaves, slight variations are not infrequent. The stages of development may be either retarded or accelerated. Under

conditions of forced leaf-fall all processes are greatly accelerated. The data in table I show that all processes, including the process of abscission, may be completed in the third pair of leaves in a period of 2 or 3 days as a result of the amputation of the blades. On the other hand, amputation of the blades of the first and second pairs of leaves before the beginning of the formation of the abscission layer inhibits its formation entirely.

Method of abscission

UNDER ORDINARY GROWING CONDITIONS.—The method of abscission has received much attention, but a critical survey of the exact changes in the cellulose and pectic compounds is wanting. An attempt to follow these changes in *Coleus* led to the discovery of certain facts which have a direct bearing upon the existing theories of the cause of abscission.

Microchemical analyses show that there is a breaking down not only of the calcium pectate of the middle lamella of the cells of the separation layer, but also of the cellulose of the secondary membrane, leaving only a thin layer of cellulose surrounding the lumen of the cells. This cellulose is first changed to pectose, which, according to CROSS (1), TOLLENS (16), and EULER (3), contains more oxygen than cellulose and probably is an oxidized form. The pectose is then further changed to pectin and pectic acid; the excess pectic acid becomes gelatinous and is no longer able to hold the cells together, and the leaf falls. The changes taking place in the walls of the xylem tubes are still to be investigated. During this process there is not a disappearance of calcium from the cell walls, but the excess of pectic acid produced renders the amount of calcium present insufficient to maintain the solidity of this portion of the cell wall. The excess pectic acid appears to come from the transformation of the pectose rather than from the breaking down of the calcium pectate of the middle lamella. Since this description differs from all previous accounts of the method of abscission, further discussion is postponed until all the facts are brought together under the topic of microchemical analysis.

FORCED LEAF-FALL.—Leaf-fall was accelerated by treatment with ethylene, amputation of the blade, and by allowing the soil

to become dry and then suddenly applying an excess of water. Under the first two treatments the leaves began to fall within 24 hours.

The rate of petiole fall after the amputation of the blade is shown in table I. At the beginning of the experiment each plant had 8 pairs of leaves and 1 blade of each pair was removed. The numbers refer to the total number of abscissed petioles at the corresponding dates.

TABLE I

RATE OF PETIOLE FALL FOLLOWING AMPUTATION OF BLADES

Plant	January					February		March							
	27	28	29	30	31	1	2	1	2	3	4	5	6	7	10
A*	1	5	....	....	....	....	6	6	....	....	....	....	....	....	6
B*	1	4	....	5	....	....	6	6	....	....	....	....	....	....	6
C†	....	....	....	....	....	....	....	....	3	4	6	....	....	6	....
D†	....	....	....	....	....	....	....	....	4	5	....	....	....	6	....
E†	....	....	....	....	....	....	....	....	4	6	6	....	....	6	....

* Blades amputated January 26; † blades amputated March 1.

A microchemical analysis of the abscission layer in all these cases showed exactly the same changes in cellulose and pectic substances as noted under ordinary conditions of growth. Furthermore, changes in oxidases, calcium in solution, and iron, to be discussed later, were the same in all cases. These facts emphasize again the need of experimental investigation before accepting the turgor pressure theory of the cause of abscission.

It is interesting to note that while the petioles usually absciss soon after amputation of the blade, there is one striking exception. If the blade is removed before the abscission layer is initiated, growth ceases throughout the entire petiole, the layer fails to develop, and the petiole is not dropped. The data in table I show that the 6 lowest petioles soon fall, but the upper 2 remain attached. This is an easy method of locating the period of formation of the abscission layer. This extreme cessation of growth in the petiole induced by artificial means has some features in common with a retardation of growth and abscission formation in the petioles of the upper leaves under natural conditions of flowering and fruiting. As a result of slowing up of growth and formation of abscission

layers in the petioles of the upper 3–5 pairs of leaves accompanying the development of the floral axis, these leaves remain on during the entire flowering and fruiting period. An investigation of the internal changes accompanying these 2 phenomena may throw some light upon abscission in general.

Organic acids as a cause of leaf-fall

WIESNER (**17–21**) cites three lines of experimental evidence as proof that the dissolution of the middle lamella is a result of the accumulation of organic acids in the aging leaves: (1) yellow leaves macerated and extracted with water when titrated were more acid than green leaves; (2) cuttings placed in 2.5 per cent oxalic acid dropped their leaves in a few days; (3) the exposed abscission surface of the petiole always gives an acid reaction to neutral red.

Abscission in *Coleus* when examined from the point of view of this theory shows several facts in disagreement and not one in its favor. Cuttings placed in non-toxic concentrations of oxalic acid showed no acceleration of leaf-fall over that of cuttings in distilled water. Cuttings in 0.0002 N oxalic acid showed slight toxic effects. The immersed part of the stem and the tips of young leaves on axillary branches became brown in color. The concentration of acid used by WIESNER was 1500 times as great, but he fails to state what plants were used and whether toxic effects were produced. Cuttings of *Coleus* in 0.0016 N oxalic acid did not show an acceleration of leaf-fall, although the toxic effects were strongly pronounced. It was further found that the plants soon became adjusted to the oxalic acid. Plants started in 0.0002 N oxalic acid were transferred every third day to a concentration of acid double that of the previous concentration. This was continued until the plants were finally placed in 0.0512 N oxalic acid. There was no acceleration of leaf-fall during the entire period. At the end of the treatment the cells of the plant were found to be filled with starch.

Similarly, potted plants infiltrated with non-toxic concentrations of oxalic acid showed no acceleration in leaf-fall. The plants were inverted under bell jars in vessels containing the various solutions, and the air was exhausted to 3 cm. of mercury. The volume of solution entering the infiltrated plant was approximately

equal to one-fourth the volume of the plant. No toxic effects were noted for concentrations of 0.0064 N oxalic acid and below. The results are given in table II.

TABLE II

SHOWING EFFECT OF DIFFERENT CONCENTRATIONS OF OXALIC ACID ON RATE OF ABSCISSION

Concentration	February													March				
	15	16	17	18	19	20	21	22	23	24	25	26	27	28	1	2	3	4	5
0.0256 N oxalic acid										2		3		4	5		6		
0.0128 " " "								2				3		5			6		
0.0064 " " "	1							2		3				4	5		6		
0.0032 " " "			1									2		5					6
0.0016 " " "										1				2	3	4	5	6	
0.0008 " " "								1		2				3		4	5	6	
0.0004 " " "										2				4	6	7		8	
Untreated										3		4		6	7				8
"			1							3		4		6		8			

The plants were infiltrated February 9, and again February 22. Leaf-fall was allowed to occur normally under greenhouse conditions. The numbers refer to the total number of leaves off at the corresponding date.

Although concentrations of 0.0128 and 0.0256 N oxalic acid showed marked toxic effects, abscission was not accelerated; concentrations between 0.04 and 0.12 N oxalic acid killed many blades without killing the petioles and stem. In such cases abscission of the petioles occurred within 2 or 3 days, just as in the case of petiole fall after amputation of the blade, or severe wounding of the blade.

Likewise direct measurements of acidity do not agree with those of WIESNER. Table IV gives the acidity for 9 different regions of the plant. Falling leaves are not so acid as green leaves. Fresh yellow leaves in the act of abscissing when titrated with NaOH, using phenolphthalein as an indicator, had an acidity equivalent to 0.0069 cc. of normal acid per gram of wet weight. Fresh green leaves collected at the same time from the same plants had an acidity equivalent to 0.0089 cc. of normal acid per gram of wet weight. In both cases the leaves were weighed as rapidly as pos-

sible after collecting, macerated in a mortar, made up to volume with distilled water, and after shaking for 30 minutes the solutions were filtered through a Buchner funnel and definite portions taken for titration. Fresh abscission layers treated in this way had an acidity of 0.0100 cc. per gram of wet weight, while that of the adjacent part of the petiole was 0.0095 cc. These two figures are not to be compared with those preceding, as the two sets of titrations were made at different times and on different plants.

If the calcium pectate were being hydrolyzed by an organic acid, one would expect to find either an increase of calcium in solution in the cells of the abscission layer or an increase of crystals of calcium compounds in these cells. Such is not the case. Neither are there any calcium oxalate crystals in the middle lamella of these cells, such as one finds when the middle lamella is broken down by adding oxalic acid to sections under the microscope.

Finally, the marked acidity of the abscission surface of a falling leaf was certainly not correctly interpreted by WIESNER. He ascribes the acidity of the abscission surface to the excretion of organic acids from the interior of the cells. This abscission surface is a continuous layer of pectic acid, formed during the abscission process, and the acidity of the abscission surface, therefore, is a result of the formation of pectic acid during abscission, and not of the escape of acids previously formed in the cells. This acidity of the middle lamella to neutral red may be seen in *Coleus* in any part of the plant, and it is increased in the walls of the abscission layer only after the formation of pectic acid during abscission.

In conclusion, therefore, neither the turgor pressure theory nor the organic acid theory proposed by WIESNER to account for the cause of leaf-fall is in accordance with the facts observed in *Coleus*.

Effect of salts on leaf-fall

According to CZAPEK (2), the membranes of plant cells are colloidal in nature, and MANGIN has shown that the middle lamella is composed of pectic acid in combination with calcium. During the process of abscission the middle lamella undergoes a chemical alteration and the pectic acid present takes up water and swells. It was expected, therefore, that salts might show either a lyotropic

effect on the intake of water by the pectic acid or a specific effect of salt formation with this acid and thus affect the course of abscission. The following anions were used in the form of their potassium and calcium salts: PO_4, SO_4, Cl, NO_3, and CNS; and the following cations in the form of their chlorides: K, Na, Ca, Ba. In all cases 0.01 normal concentrations were used. The plants were treated by infiltration, by placing cuttings directly in the solutions, and by adding the salts to the soil. Abscission was slightly accelerated by treating the plants with 4 parts of ethylene per million of air. In all cases the results were the same. Neither lyotropic nor specific effects were noted. Similarly, concentrations of potassium and calcium chlorides between 0.04 and 0.00016 normal showed no marked effect.

The experiment was repeated under conditions of rapid acceleration of abscission by treating the plants with 700 parts of ethylene per million of air. The results which agree with those above are summarized in table III.

TABLE III

SHOWING EFFECT OF SALTS ON ABSCISSION

CONCENTRATION	FEBRUARY 12	FEBRUARY 15		FEBRUARY 14		FEBRUARY 13	
	5 P.M.	9 A.M.	5 P.M.	9 A.M.	5 P.M.	9 A.M.	5 P.M.
0.01 N KH_2PO_4		1		3	5		5
" " K_2SO_4			2	3	4		4
" " $CaSO_4$			5	6			6
" " KCl			3	4	5		5
" " KNO_3		2	3	5			5
" " $Ca(NO_3)_2$				2		4	4
" " KCNS		1		3		4	4
Untreated		2			3	4	4
"	2	5	6				6
"				2		3	3
"		1		4		6	6
"		3		4		6	6
0.01 N KCl			3	4	5		5
" " NaCl			3	4	4		4
" " $CaCl_2$				4	4		4
" " $BaCl_2$			2	4	4		4

Cuttings in solutions of the different salts gave similar results. The great diversity of the checks noted in this table is very unusual.

The failure of calcium to show a specific effect was unexpected. Later work, however, has thrown some light upon the matter, and it will be discussed under microchemical analysis.

Oxygen pressure and leaf-fall

MOLISCH (**14**) grew plants half submersed in water and found that the aerial portions dropped their leaves sooner than the submersed portions. From this fact he concluded that low oxygen pressure retarded leaf-fall. This surmise proved to be correct. *Coleus* plants grown in a hydrogen atmosphere under bell jars with only sufficient oxygen to maintain a slow growth retain their leaves much longer than plants in normal air. Under conditions of the experiment the plants in normal air usually retain 8 pairs of leaves. In the hydrogen atmosphere the plant retained 11 pairs of leaves. Inception of decay at the base of the stem destroyed the experiment at this point. Similarly, petiole fall, after amputation of the blade, is greatly retarded in very low concentrations of oxygen. Plants grown in 0.1 normal oxygen pressure showed no retardation of leaf-fall. Whether the effect of oxygen in such cases is that of an essential factor influencing the general metabolism of the plant, or of a formative factor influencing directly the oxidase activity in the abscission layer, or of both acting simultaneously, is a problem still to be investigated. Likewise a critical investigation of the possibility of a double effect of carbon dioxide on leaf-fall might throw more light upon the causes underlying abscission.

Macrochemical analysis

In order to follow the chemical changes leading up to abscission, both macrochemical and microchemical methods of analysis were employed. About 2500 plants grown under greenhouse conditions were used for the macrochemical analysis. These plants were all grown at the same time under the same conditions, and collection of material was made at the same time each day. Series C was collected between February 17 and March 3, collections being taken from day to day as the lower leaves began to absciss. Series D and E were collected from these same plants on March 3 and 4.

Material when collected was placed in 70–80 per cent alcohol and heated to 70°C. for one hour to destroy enzymatic activity.

The material was then extracted with alcohol and ether. The residue was dried and analyzed for polysaccharides, calcium, and oxalates. The alcohol-ether extract was evaporated to dryness on a steam bath and then extracted with water at 70°C. The filtrate of this aqueous extract was analyzed for reducing and non-reducing sugars; ammonia, amino acid, and nitrate nitrogen; calcium in solution, and acidity.

Table IV gives a summary of an analysis of 9 different regions of the plant. Series C represents leaves in the act of abscissing, series E represents leaves at the time of the formation of the abscission layer, and series D represents leaves intermediate between these two points. Collection E_1 represents approximately 5 mm. of the abscission end of the petiole, collection E_2 an equal portion of the adjacent part of the petiole, and collection E_3 a portion of the blades. In like manner, collections C_1, C_2, and C_3 and collections D_1, D_2, and D_3 represent these same three regions in their respective series.

Attention should be called to the fact that while collections C_1, D_1, and E_1 represent the abscission end of the petiole, they do not represent the abscission layer only. In no case does the abscission layer represent more than about 5 or 6 per cent of the portion of the petiole taken. In collection C_1 it represents still less, probably not more than 2 per cent, as in the abscissing leaf the petiole retains only about one-third of the abscission layer, the remaining two-thirds being attached to the stem.

It is evident that chemical changes in the abscission layer might be overshadowed by the remaining 95 per cent of the collection, and even more so in collection C_1 than in collections D_1 and E_1. This is especially true of the nitrates, which are frequently confined almost entirely to the abscission layer and are more abundant in this layer at the time of abscission than at any other time, although the figures in the table might lead one to think they were most abundant a short time before abscission. As a matter of fact, a large percentage of the nitrates in the abscission layer of collection C_1 were left in the part of the abscission layer remaining attached

TABLE IV

Collection	Percentage dry weight	Percentage of dry material			Expressed as percentage of total dry weight										Total acidity as normal acid (in cc.) per grain of wet weight
		Water soluble	Alcohol-ether soluble	Alcohol-ether but not water soluble	Total carbohydrates	Polysaccharides	Non-reducing disaccharides	Reducing substances	NH_3 nitrogen	Amino acid nitrogen	NO_3 nitrogen	Total calcium	Calcium in solution	Calcium not in solution	Oxalates as anhydrous oxalic acid	
E1	3.94	28.92	31.55	2.63	11.89	11.44	0.0	0.45	0.063	0.106	0.900	3.06	0.17	2.89	1.97	0.0068
E2	3.95	28.76	32.04	3.28	13.23	12.33	0.0	0.90	0.048	0.033	0.042	3.20	0.20	3.00	2.07	0.0079
E3	7.43	13.23	22.76	9.53	17.04	15.09	0.13	1.82	0.020	0.056	0.140	2.18	0.06	2.12	1.34	0.0057
D1	3.61	30.85	35.39	4.54	14.02	11.88	0.40	1.74	0.076	0.097	2.820	3.07	0.37	2.70	1.41	0.0073
D2	3.29	.....	33.32	.....	14.03	12.03	0.0	2.00	0.072	0.057	0.180	3.42	0.33	3.09	1.31	0.0058
D3	6.74	17.54	26.00	8.46	24.00	19.70	0.0	4.27	0.009	0.058	0.037	2.52	0.09	2.43	1.47	0.0100
C1	4.33	25.56	45.96	20.40	13.21	10.95	0.0	2.26	0.030	0.080	1.670	2.32	0.12	2.20	2.07	0.0069
C2	3.33	29.44	32.97	3.53	15.02	10.42	0.0	4.60	0.036	0.078	0.950	3.06	0.21	2.85	1.70	0.0055
C3	5.06	19.85	27.34	7.49	15.82	10.88	0.58	4.35	0.024	0.072	0.036	3.61	0.12	3.49	2.02	0.0065

to the stem, and therefore are not included in the analyses. The data in the table, therefore, represent only the general chemical changes during the life of the leaves, while the detailed chemical changes occurring in the abscission layer itself will be given under microchemical analysis. Should one desire to make a macrochemical analysis of the abscission layers alone in *Coleus* no less than 40,000 plants would be needed.

The data in table IV show an increase in dry weight in the abscission end of the petiole at the time of abscission; also an increase in alcohol-ether soluble material, but no increase in water soluble material. The significance of these changes is uncertain. In the older petioles there is a slight decrease in polysaccharides and an increase in reducing substances. There is no increase in ammonia and amino acids, as might be expected if the protoplasm were breaking down. Oxalates and total calcium remain fairly constant, but there is a slight decrease in the amount of calcium in solution and in the acidity. A more detailed discussion of the chemical changes in the abscission layer is given under microchemical analysis.

In the older blades there is a decided decrease in the amount of accumulated starch at the time of abscission, but the amount of reducing substances remains fairly constant. Attention has already been called to the fact that the formation of the abscission layer is completed while the leaf is still in an active photosynthetic condition. Both photosynthesis and the translocation of foods continue for several days or weeks later. The data clearly show that the presence of the abscission layer does not prevent the movement of water and foods between leaf and stem.

Microchemical analysis

A microchemical investigation of *Coleus* showed a striking localization of physical and chemical changes in the abscission layer shortly before and at the time of abscission. The formation of the abscission layer usually in the third pair of leaves and the occurrence of abscission usually in the eighth pair of leaves (when the plants are grown in 4-inch pots in a greenhouse) make it possible to study the whole history of the abscission layer by investigation of only 6

pairs of leaves in each plant. The fact that the leaves are opposite is also of advantage. Abscission of the pair may occur simultaneously, or one of the pair may absciss long before the other begins, or it may occur at any stage in between. Since both abscission layers at each node may readily be obtained in a single free-hand section, it is possible to contrast all stages of abscission under exactly the same treatment. A study of the changes induced by forcing abscission in one of the leaves at each node is likewise facilitated.

The investigations completed include a study of the distribution and amount of nitrates, carbohydrates, oxidases, iron, manganese, calcium in solution, and oxalates.

NITRATES.—The data in table IV show a great increase of nitrates in the abscission end of the petiole as compared with the remainder of the leaf. Furthermore, the nitrates in this part of the petiole are least abundant at the time of formation of the abscission layer and most abundant a short time before leaf-fall. As already noted, these figures cannot be taken to represent the percentage of nitrates in the abscission layer. Microchemical tests show some interesting variations. In many plants the increase in nitrates is confined almost entirely to the abscission layer, while in others the petiole or the neighboring part of the stem may also show a like increase. In all cases studied there is an increase in nitrates in the abscission layer just before and at the time of abscission. In some plants this increase is gradual from the time of the formation of the abscission layer to the time of abscission. In other cases only traces of nitrates appear in the abscission layer until a short time before abscission, when they increase rather suddenly.

CARBOHYDRATES.—The data in table IV show that the free reducing sugars, like the nitrates, increase in the abscission end of the petiole with the increase in the age of the leaves, but, unlike the nitrates, they are less abundant in this part of the petiole than in the remainder of the leaf. This correlation of the amount of reducing sugars and the age of the tissue is still more striking when studied microchemically. From the terminal bud to the oldest leaves there is a gradual increase in reducing sugars in both stems and leaves. This increase is initiated last in the abscission layer.

As a result the abscission layer has a lower percentage of reducing sugars throughout its entire history than the adjacent regions of the petiole. This difference is most marked in the fifth and sixth pairs of leaves near the close of the formation of the abscission layer, but it is still quite evident at the time of abscission. In the cell walls of the abscission layer the change in form of the carbohydrates is still more pronounced and significant. During the process of abscission the first evident change in the cell walls is a conversion of cellulose of the secondary cell membranes to pectose. The second step is a conversion of some of this pectose to pectic acid and pectin. This is followed by the breaking down of the middle lamella of calcium pectate and the separation of the cells. The changes from cellulose to pectose can readily be followed by differential staining and crystallization methods, and by solubility tests. The evidence of the conversion of pectose to pectin and pectic acid is based upon solubility tests. Pectin is soluble in water, pectic acid is insoluble in water but soluble in dilute alkalies, while pectose is insoluble in both water and dilute alkalies. When an abscission layer at the time of abscission is treated with 3 per cent ammonium or potassium hydroxide or with 5 per cent sodium carbonate, the free pectic acid is dissolved. If the walls are then again examined a considerable portion of the secondary membrane, bordering the middle lamella which is still intact, is seen to have disappeared. A discussion of the changes in the calcium pectate is postponed until all the remaining facts have been stated.

OXIDASES.—In the stem and petioles oxidases are found in the epidermal and phloem tissues. In the abscission layer oxidases are found in all tissues except the xylem. Not only is this distribution peculiar to this region, but the increase in oxidases with the age of the abscission layer is also quite pronounced. Quantitative tests of the increase in oxidative activity are still to be made.

IRON.—Slight traces of iron (Fe^{+++}) are usually found throughout the plant, especially where chlorophyll is present. It is most abundant in the xylem tubes and in the epidermal region until a few hours before abscission, when it becomes extraordinarily abundant in the cells of the abscission layer. The path of diffusion of

the iron leading to its accumulation in the abscission layer has not been traced. No manganese was found.

CALCIUM AND OXALATES.—The data in table IV show no marked difference in the distribution of oxalates in the leaves. Only occasional crystals of calcium oxalate are found in the cells, and none are found in the cell walls of the abscission layer at any time. Likewise the total calcium has a fairly constant distribution throughout the plant, but slight variations of the calcium in solution are to be noted. The most striking and significant changes of the amount of calcium in solution, however, are shown by microchemical tests. Treatment of sections with 50 per cent sulphuric acid or with 3 per cent oxalic acid or ammonium oxalate show an abundance of calcium in solution in all living cells of the petiole except those of the abscission layer at the time of abscission. The crystals of calcium sulphate or of calcium oxalate obtained by these treatments were very numerous in the cells of the abscission layer before the time of abscission, the latter averaging 30 crystals per cell, while during abscission only an occasional crystal was obtained. This decrease of calcium in solution is not always confined to the abscission layer, but breaks off rather abruptly in the first few layers of cells of the adjacent region of the petiole. In some cases cells not more than 5 cell layers distant from the line of cleavage showed no decrease in the number of crystals. These facts show that the calcium in solution in the abscission layer disappears during abscission, and it should be further stated that the disappearance takes place in the first stages of the process.

Summary of microchemical analysis

1. A pronounced increase in nitrates always occurs in the abscission layer at the time of abscission. This increase may be gradual, extending over the entire life-history of the abscission layer, or it may appear somewhat suddenly a short time before abscission.

2. A gradual increase in the amount of reducing sugars accompanies the aging of leaves and stem. This increase is initiated last and is least pronounced in the abscission layer.

3. During the process of abscission the cellulose of the secondary membrane of the cell walls of the abscission layer is converted into

pectose. This pectose is further transformed into pectic acid and pectin. The final stage is the breaking down of the calcium pectate of the middle lamella.

4. Oxidases are present in the epidermal and phloem tissues in both stems and petioles. In the abscission layer they are present in all tissues outside of the xylem, and increase in amount with the age of the abscission layer.

5. Slight traces of iron may be found in practically all parts of stem and petioles, but shortly before abscission there is a sudden accumulation of iron in the abscission layer.

6. The amount of oxalates remains fairly constant throughout the entire life of the leaves. There is no evidence of an increase of calcium oxalate crystals in the cells of the abscission layer at the time of abscission, nor are there any crystals of calcium oxalate in the walls of these cells.

7. Calcium in solution is abundant in all living cells of the plant except those of the abscission layer at the time of abscission, where it practically disappears.

Discussion

According to TOLLENS (**16**), pectose is an oxidized cellulose of the composition $9(C_6H_{10}O_5)-C_6H_{10}O_6$. The first step in the breaking down of the cell walls in abscission in *Coleus* is evidently one of oxidation of cellulose. This process is possibly a result of the accumulation and subsequent activity of oxidases in the abscission layer, and also of the catalytic action of iron on these oxidases. This may be merely an acceleration of the conversion of cellulose into pectose which ordinarily goes on in cell walls of plants with increasing age. Cellulases may play a part in this process, but the question is still to be investigated. EULER (**4**) succeeded in isolating a cellulase in a fungus, *Merulius lacrimans*, which was capable of altering cellulose, but cellulases in higher plants are still unknown. CZAPEK (**2**) and EULER (**3**) have called attention to the fact that our knowledge of cellulases is very limited.

The pectose formed from the cellulose is in turn readily transformed to pectic acid and pectin, and in this process the catalytic action of iron may again play an important rôle. Whether acids

and pectic enzymes also play a rôle in these changes is uncertain. Hydrolytic action may underlie some or all of these changes, but this must remain an open question until the molecular composition of these compounds is definitely known. At any rate, the transformation of the cellulose and pectose leads to the formation of an excess of pectic acid in the cell walls of the abscission layer.

The most important question still open is the cause of the final breaking down of the calcium pectate of the middle lamella. There appear to be but two possibilities. Either the calcium ion of the pectate is captured by some anion, liberated in the cells of the abscission layer, and held in solution or precipitated, thus freeing the pectic acid, or the breaking down of the cellulose and pectose may lead to such an excess of pectic acid that the available calcium is no longer able to hold a sufficient proportion of the pectic acid as a salt, and thus maintain the solidity of the middle portion of the cell wall.

The fact that calcium is not found in solution in the cells of the abscission layer, nor in crystalline forms either in the cells or in the cell walls, is decidedly against the first view, which is simply WIESNER'S organic acid theory stated in slightly different terms and which has already been discussed in detail.

The second view is more easily understood when we recall the well known law of physical-chemical equilibrium. As soon as an excess of pectic acid is present in contact with the calcium pectate of the middle lamella there is undoubtedly a diffusion of calcium ions from the middle lamella and a diffusion of pectic acid into the middle lamella until an equilibrium of distribution of the two ions is established. A critical proportion of pectic acid to calcium would be reached in the middle lamella when the excess pectic acid breaks the continuity of the calcium pectate layer. This second view has the further advantage of being in accordance with all the experimental facts so far known, particularly the formation of excess pectic acid in the cell walls and the paucity of calcium in solution in the cells of the abscission layer.

The fact already stated, in the discussion of calcium, that there is an abundance of calcium in solution in cells within 5 cell layers of the line of cleavage in abscission, indicates either that the process

of abscission is a very rapid one or that the diffusion of calcium from cell to cell is a very slow one. This fact also explains why the addition of calcium salts, already discussed, showed no specific effects on the rate of leaf-fall as the rate of diffusion of the calcium ions through the cells would again appear as a limiting factor.

Extensive comparative investigations of abscission in the light of facts discovered in *Coleus* are still to be made. TISON'S statement that in general the secondary membranes also are altered in abscission indicates that these processes may be rather general, particularly since the reports of HANNIG and LLOYD of a similar alteration in the abscission of floral organs. Investigation of the more fundamental factors underlying the ultimate chemical changes discussed in this paper must be made before general conclusions of the causes leading up to abscission can be drawn. The significance of the presence of an abundance of nitrates in the abscission layer at the time of abscission is uncertain. Their ability to affect the water holding capacity of colloids and similar effects of other ions which are changing in concentration in this region may influence the permeability of the cell membranes of these cells, a question that has not yet been touched upon experimentally.

Conclusion

Abscission of leaves in *Coleus Blumei* is a result of the conversion of cellulose into pectose, which is further transformed to pectin and pectic acid, leading to the formation of an excess amount of pectic acid over that of the available calcium sufficient to maintain the solidity of the middle lamella of the cell walls of the abscission layer. These processes are possibly initiated and probably accelerated by the presence of oxidases and ferric ions, both of which accumulate in the abscission layer.

Microchemical methods employed

In the microchemical study color reactions were used for orientation. These were followed by specific chemical reactions and solubility tests. A brief outline of the tests made for each substance follows. Details of these reactions may be found in recent microchemical texts.

CELLULOSE.—(1) Chlorzinc iodide: blue color; (2) hydrocellulose reaction: blue color with iodine after treatment with 75 per cent sulphuric acid; (3) solubility: insoluble in dilute acids and alkalies, soluble in copper-oxide-ammonia; (4) crystallization: dissolve in copper-oxide-ammonia, wash with ammonia and water; colorless sphaero crystals or spiculate crystal clusters appear within the cells; (5) crystal reactions: insoluble in dilute acids and alkalies, soluble in copper-oxide-ammonia and sulphuric acid; blue color with chlorzinc iodide; (6) membranes of cellulose exhibit double refraction in polarized light.

PECTIC COMPOUNDS IN GENERAL.—(1) Ruthenium red: red color; (2) methylene blue: violet color; (3) membranes of pectic compounds do not exhibit double refraction in polarized light.

PECTOSE.—(1) Insoluble in copper-oxide-ammonia, dilute alkalies, ammonia, and alkali carbonates; (2) converted into pectic acid and pectin when gently heated with 2 per cent hydrochloric acid for 30 minutes. These latter substances are readily dissolved by 2 per cent potassium hydroxide or 5 per cent sodium carbonate, leaving the cellulose membrane intact.

PECTIC ACID.—(1) Soluble in dilute alkalies, ammonia, and alkali carbonates; (2) insoluble in water.

PECTIN.—Soluble in water.

CALCIUM PECTATE.—(1) Hydrolyzed by 2 per cent hydrochloric acid: calcium chloride is formed and pectic acid set free; (2) 3 per cent oxalic acid or ammonium oxalate: calcium oxalate crystals are formed, pectic acid set free; (3) 5 per cent sulphuric acid: calcium sulphate crystals formed, pectic acid set free.

CALCIUM.—(1) Two per cent oxalic acid: calcium oxalate crystals; (2) 5 per cent sulphuric acid: calcium sulphate crystals.

LIGNIN.—Phloroglucin-HCl reaction: red violet color.

SUBERIN.—(1) Sudan III or Scharlach R: red color; (2) insoluble in copper-oxide-ammonia; (3) phellonic acid reaction.

FRUCTOSE.—(1) Fluckiger's reaction: yellowish-red precipitate of cuprous oxide at once without heating; (2) phenylhydrazine reaction: yellow osazone crystals formed in 6–8 hours; (3) methylphenylhydrazine reaction: insoluble osazone; crystals formed in 15 minutes if preparation is heated, after 24 hours at room temperature.

GLUCOSE.—(1) Fluckiger's reaction: yellowish-red precipitate of cuprous oxide after heating 1–2 minutes; (2) phenylhydrazine reaction: yellow osazone crystals formed after about 24 hours.

SUCROSE.—Remove fructose and glucose. Invert with hydrochloric acid. Test for glucose and fructose as preceding.

NITRATE.—(1) Diphenylamine sulphuric acid reaction: blue color slowly changing to brown-yellow; (2) brucin-sulphuric acid reaction: red color.

OXIDASES.—Benzedine reaction: blue or purple precipitate if tissue is acid, soon changing to brown; brown precipitate at once if tissue is neutral or alkaline.

IRON.—(1) Berlin blue reaction: sections in 2 per cent solution of potassium ferrocyanide 15 minutes, add a drop of 2 per cent hydrochloric acid. A dark blue precipitate indicates the presence of ferric ions. Similarly a red color with potassium ferricyanide indicates the presence of ferrous ions; (2) sodium thiosulphate: red color.

MANGANESE.—Sections in 0.1 per cent hydrochloric acid, add 0.5 per cent sodium ammonium phosphate and ammonia vapor: ammonium manganese phosphate crystals, brown color in a 2 per cent solution of potassium permanganate.

MALIC ACID.—(1) Silver nitrate: sphaero crystals of silver nitrate, soluble in ammonia; (2) lead oxide: lead malate crystals; (3) sublimation: concentrated sulphuric acid, heat to 130°C.; slight charring.

OXALIC ACID.—(1) Uranium acetate: large yellow crystals of uranium oxalate; (2) strontium nitrate: strontium oxalate crystals; (3) ferrous phosphate: yellow precipitate of ferrous oxalate.

AMINO ACIDS.—Crystallization: treat sections with absolute alcohol, crystals of amino acids; (1) compare with known crystal form; (2) specific reactions.

TYROSINE.—Millon reaction: red color.

ARGININE, HISTIDINE.—Picrolonic acid: yellow crystalline precipitate.

LEUCINE.—Sublimation at 170°C.

ASPARAGINE, GLUTAMINE.—Quinone: red color.

The writer wishes to express his gratitude to Dr. WILLIAM CROCKER and Dr. SOPHIA H. ECKERSON for numerous suggestions and criticisms during the progress of the work.

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INTERRELATIONSHIPS OF THE TAXINEAE

MARY C. BLISS

(WITH PLATES I, II)

In considering the Taxineae it is interesting to note the taxonomic position to which this subtribe has been assigned at various periods in the history of the classification of the conifers. ENGLER and PRANTL (**4**) in 1889 placed it at the top of the group; PENHALLOW (**6**) in 1907 placed it at the bottom of the group; COULTER and CHAMBERLAIN (**1**) in 1901 regarded the subtribe as the most primitive of the conifers and placed it at the bottom, but in 1910 (**2**) shifted its position to the top of the group as the most modern. These facts show clearly that the family is a difficult one to interpret, and the difficulty is due in part to the fact that the Taxineae combine at the same time extreme simplification and specialization.

The argument presented by PENHALLOW as evidence for his theory that the Taxineae are the most primitive of the conifers is based on the progressive development of the resin canals in *Pinus* and *Picea* from the isolated resin cells of *Podocarpus* "by various phases of aggregation." In *Taxus* and *Torreya* of the Taxineae, which he investigated, PENHALLOW states that resin cells are entirely wanting. Isolated resin cells occur in abundance in *Podocarpus* of the Taxineae. In the true Coniferae isolated or aggregated resin cells are characteristic of all the genera except *Picea* and *Pinus*, where they are replaced by resin passages, of which the aggregations of resin cells form an essential part. From the genera *Taxus* and *Torreya*, characterized by the absence of resin cells, PENHALLOW traces a series through *Podocarpus*, where resin cells are scattered, to genera of the Coniferae, where first, as in *Taxodium* and *Libocedrus*, the resin cells are arranged in well defined zones as well as scattered, to resin sacs in *Abies* and *Sequoia*, to resin passages with constrictions in the canal in *Larix*, *Pseudotsuga*, and *Picea*, to the resin passages without constrictions, as in *Pinus*.

Those who hold that the Taxineae represent a modern group in the evolution of the conifers interpret the facts already stated in PENHALLOW'S argument as evidence of an entirely different progression. Starting with the genera in which the resin canal is highly specialized and resin cells wholly lacking, as in *Pinus*, they trace a series in which there is a gradual reduction of the resin canal, to aggregations of resin cells as in *Taxodium*, to scattered resin cells as in *Sequoia* and *Podocarpus*, to entire absence of resin cells as in *Taxus*.

As evidence of the fact that resin canals represent a primitive condition in the conifers, JEFFREY'S work on the genus *Sequoia* may be cited. If the presence of resin canals were evidence of modern development, we should expect to find them in the mature and more progressive parts of the plant, but in *Sequoia gigantea* JEFFREY (5) found the resin canals only in the first annual ring in the stem, in the ovulate strobilus, and in the leaf traces of very vigorous leaves of adult trees. In *Sequoia sempervirens* the resin canals were wholly lacking in these regions, but in injured stems and roots of both *S. gigantea* and *S. sempervirens* resin canals were present. In the case of *Sequoia*, then, the presence of resin canals represents a primitive condition in the conifers, retained only in the more conservative regions of the plant in *S. gigantea*, and wholly absent in *S. sempervirens*. A reversion to the ancestral condition in both species may be induced by injury.

According to this later view of the position of the Taxineae as contrasted with that held by PENHALLOW, we have a series of genera starting with *Pinus* as a representative of the most primitive group in which resin canals are normally present, proceeding through *Sequoia* as a type of a group in which resin canals are not normally present in the vegetative axis, until we come to *Podocarpus*, a representative of a group in which resin canals are never present.

In this connection it is important to note that in those groups in which resin canals are normally absent the secretion of resin is carried on by resin parenchyma cells. These resin cells are characteristic of the Taxodineae, Cupressineae, and Podocarpineae. We should expect as the logical outcome of this gradual reduction and simplification of resin secreting structures the final passing out

of the resin parenchyma cells in the more modern types. This question will be taken up later when we consider the genera within the group Taxineae.

Much of the controversy in regard to the position of the Taxineae has been based on the character of the gametophytes and reproductive structures, especially the ovulate cone and the method of development of the proembryo. The evidence which I have to offer is derived wholly from the study of the anatomical structure of the stem and root of various genera in the group.

There are included in the family Taxaceae of ENGLER and PRANTL (4) the genera *Phyllocladus*, *Ginkgo*, *Cephalotaxus*, *Torreya*, and *Taxus*. In the most recent classification of the group by COULTER and CHAMBERLAIN (2) *Phyllocladus* is included in the Podocarpineae, *Ginkgo* has been put in a family by itself, and the Taxineae include in addition to *Taxus*, *Torreya*, and *Cephalotaxus*, the doubtful New Caledonian genera *Acmopyle* and *Polypodiopsis*. Turning our attention to the 3 accepted genera of the group, *Cephalotaxus*, *Torreya*, and *Taxus*, I shall attempt to show that the Taxineae are the most modern group of the conifers, that *Cephalotaxus* is the most primitive genus of the subtribe and most nearly related to the Podocarpineae, that *Torreya* is intermediate, and that *Taxus* is the most modern genus of the family and represents, so to speak, the last word in the evolution of the conifers.

If we examine a transverse section of the stem of *Podocarpus totara*, we note the presence in great abundance of resin parenchyma cells (fig. 1). These parenchyma cells are even more evident in the longitudinal section of the stem (fig. 2) as cells which stain densely with haematoxylin due to the presence of resin. These cells are narrower than the tracheids and are characterized by thin walls, by the absence of pits, and end walls at right angles to the long axis of the cell.

A section of the stem of *Cephalotaxus drupacea* (fig. 3) presents a very similar appearance to the stem of *Podocarpus*. That the resin parenchyma cells are widely distributed throughout the annual rings of the stem is evident from a consideration of the low power photograph (fig. 4). The location of these cells is shown by the deep staining of the resinous contents.

The root of *Cephalotaxus drupacea* (fig. 5) shows the presence of resin parenchyma in even greater abundance, and this is the condition we should expect to find, since the root is the more conservative organ of the plant and would retain more fully the primitive or ancestral characteristics of the plant.

The stem of *Torreya taxifolia* presents a very different appearance from the stem of *Podocarpus* and *Cephalotaxus* already considered. Resin parenchyma cells are present throughout the annual ring, but they are much less abundant than in the other stems. The distribution of the cells may be seen in the transverse section (fig. 6), and the character of the cells is shown very clearly in the longitudinal section (fig. 4).

As previously stated, PENHALLOW did not find resin cells in any of the species of *Taxus* or *Torreya* which he investigated, and DEBARY (3) also states that all investigated species of the Coniferae, with the single exception of *Taxus*, have resin passages or resin reservoirs. As a result of my investigation, it is clearly evident that resin parenchyma is present in *Torreya taxifolia*, one of the species investigated by PENHALLOW.

If we examine a transverse section of the stem of *Taxus brevifolia* (fig. 8), we note the complete absence of resin parenchyma cells. A longitudinal section of the same stem (fig. 9) shows even more clearly that the vascular cylinder consists simply of thick-walled tracheids, with numerous bordered pits, and the characteristic spiral thickenings. So far then the condition in *Taxus* tallies with the investigations of PENHALLOW and DEBARY; but if we examine a transverse section of the root of *Taxus cuspidata* (fig. 10) we note the presence of resin parenchyma diffused throughout the annual ring. A higher magnification of a portion of the root is shown in fig. 11. Here the resin parenchyma cells are very conspicuous. A longitudinal view of the same root also shows a view of these parenchyma cells very clearly (fig. 12). The root of *Taxus baccata* also shows the presence of resin parenchyma diffused throughout the annual ring.

Although in the normal stem of the species of *Taxus* investigated there was no resin parenchyma present, a wounded stem of *T. baccata* showed very clearly an extreme development of these cells.

The location of the cells can be determined easily in the transverse section of the stem, due to the fact that the walls stain a deep blue with haematoxylin, and in the longitudinal section the characteristic shape of the parenchyma cells as contrasted with the tracheids make them easily recognizable.

We find then in the normal stem of *Taxus* the condition which we should expect as the ultimate result of the gradual reduction of resin canals, namely, resiniferous parenchyma which finally completely disappears except in the case of conservative organs.

There are three important principles of evolution which have to be considered in working out the ancestry of any group of plants, namely, the principles of recapitulation in the development of the embryo and seedling stages of the plant; retention of ancestral characters in the more conservative regions of the plant, as the root, leaf, and reproductive axis; and reversion to ancestral conditions through injury.

The first of these principles I have not been able to demonstrate, as I did not have access to the seedling stages of the genera investigated. The principle of the retention of ancestral characters in the most conservative organ of the plant is very clearly evidenced in the root of *Taxus cuspidata* by the presence of resin parenchyma cells which have entirely passed out of the stem; and finally the presence in abundance of resin parenchyma in the wounded stem of *Taxus baccata* seems to show clearly that we have in this instance a reversion to the ancestral condition.

From a consideration of these facts the evidence seems to justify the conclusion that the Taxineae are the most modern group of conifers; that of the Taxineae, *Cephalotaxus* is the most primitive, in most nearly resembling *Podocarpus* in the abundance of resin parenchyma; that *Torreya* is the intermediate genus in the group, as shown by the reduction of resin parenchyma, especially in the stem; and that *Taxus* is the most modern genus in the group, since we find here entire absence of resin parenchyma in the stem, although it is retained in the root.

Summary

1. Resin parenchyma is present in abundance in the stem and root of *Cephalotaxus drupacea* and shows clearly its close relationship

to the Podocarpineae, a family in which resin parenchyma is universal.

2. Resin parenchyma is present in less abundance in the stem of *Torreya taxifolia*, showing in this respect an intermediate position between *Cephalotaxus* and *Taxus*.

3. Resin parenchyma is wholly absent in the normal stem of *Taxus brevifolia*, showing that this genus is the most modern one of the group.

4. Resin parenchyma in the root of *Taxus cuspidata* and *T. baccata* and in the wounded stem of *T. baccata* indicates the ancestral condition in this genus.

5. The Taxineae represent a modern group of conifers, as shown by the gradual reduction and final passing out of resin parenchyma in the more progressive organs.

This investigation was carried on in the laboratories of Plant Morphology at Harvard University under the direction of Dr. E. C. JEFFREY, and I wish to express my thanks to him for his invaluable aid in the work and for the many courtesies extended to me during the year spent in his laboratory.

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EXPLANATION OF PLATES I, II

PLATE I

FIG. 1.—Transverse section of wood of stem of *Podocarpus totara*, ×250.

FIG. 2.—Longitudinal radial section of wood of stem of same, ×250.

FIG. 3.—Transverse section of wood of stem of *Cephalotaxus drupacea*, ×250.

FIG. 4.—Same as fig. 3, ×40.

FIG. 5.—Transverse section of wood of root of *Cephalotaxus drupacea*, ×250.

FIG. 6.—Transverse section of wood of stem of *Torreya taxifolia*, ×250.

PLATE II

FIG. 7.—Longitudinal radial section of wood of stem of *Torreya taxifolia*, ×250.

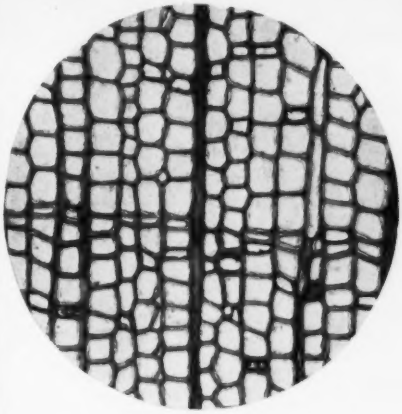
FIG. 8.—Transverse section of wood of stem of *Taxus brevifolia*, ×250.

FIG. 9.—Longitudinal radial section of wood of stem of *Taxus brevifolia*, ×250.

FIG. 10.—Transverse section of wood of root of *Taxus cuspidata*, ×30.

FIG. 11.—Upper portion of same, more highly magnified, ×125.

FIG. 12.—Longitudinal radial section of wood of root of *Taxus cuspidata*, ×125.



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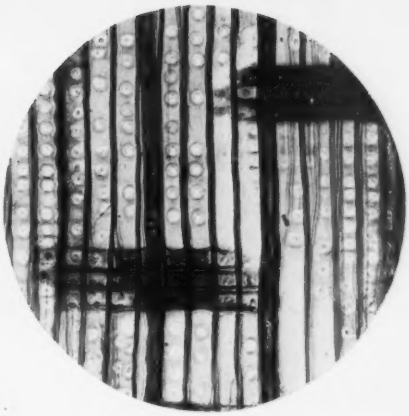

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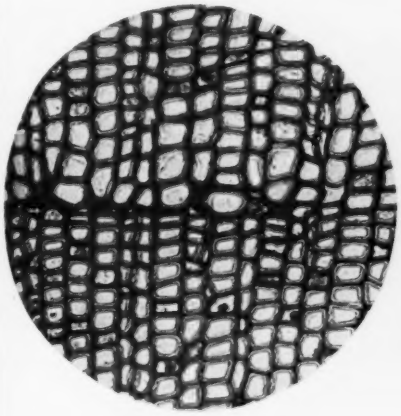

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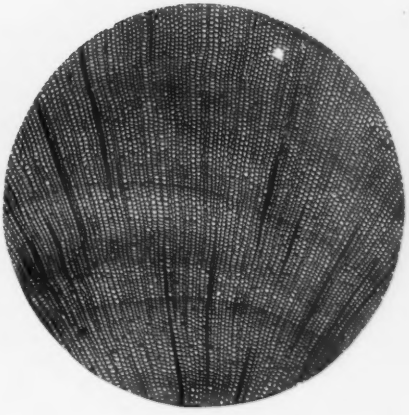

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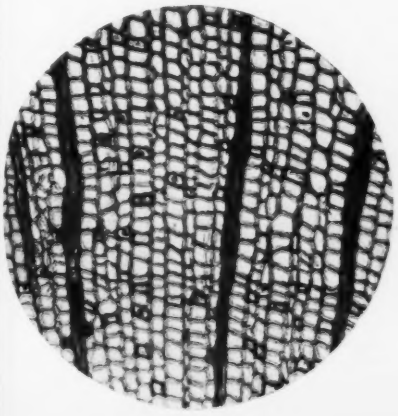

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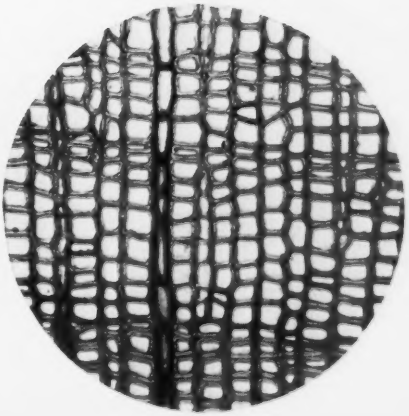

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BLISS on TAXINEAE

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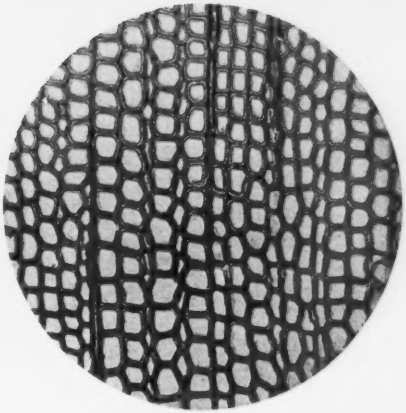

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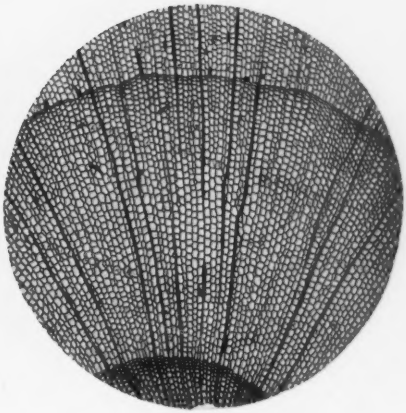

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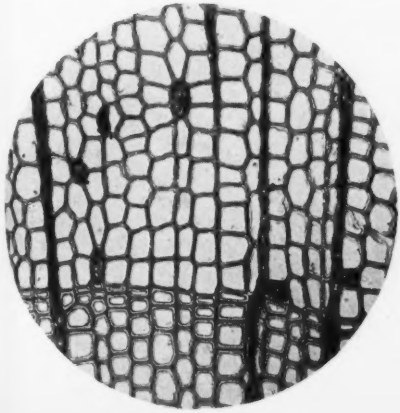

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BLISS on TAXINEAE

SIGNIFICANCE OF RESINOUS TRACHEIDS

SAMUEL J. RECORD

(WITH FIVE FIGURES)

The occurrence of resinous tracheids in gymnosperms has been noted by PENHALLOW[1] in the woods of certain species of *Cordaites*, *Araucaria*, *Dammara*, and a few representatives of the higher Coniferales, namely, *Pinus albicaulis*, *P. parviflora*, *Abies Fraseri*, and *A. grandis*. Such tracheids do not differ structurally from other tracheids, but are distinguished by their resinous contents. The resin within them is localized and usually extends across the cavity to form an imperforate septum or plate, which, in unstained sections, may give the cell the appearance of being structurally septate.

PENHALLOW figures the common form of these plates in *Dammara australis*. Figs. 1–3 illustrate the resinous tracheids in the wood of *Pinus albicaulis*, showing the characteristic form of the resin masses (*RPl*) and their association with the medullary rays. The resinous contents of the latter are omitted in order not to obscure the structure. It will be noted that there is considerable difference in the thickness of these plates, which are invariably thinnest in the middle, and not infrequently ruptured there. By comparison with PENHALLOW's drawings it will be seen that the location, form, and distribution of the resin masses in the two species are identical.

The close association of the rays with the resin plates in the tracheids clearly indicates the origin of the resin, which in some cases can be seen in the form of globules on the outside of the pit membrane of the parenchyma cell. When enough has exuded to form contact with the opposite wall of the tracheid, the surface tension of the liquid and the attraction of the cell wall cause it to assume a double concave form like a drop of water in a small glass

[1] PENHALLOW, D. P., A Manual of the North American gymnosperms. Boston: Ginn & Co. 1907 (pp. 53–58).

tube. Sometimes there is enough resin to fill the tracheid for a considerable portion of its length, at others only enough to produce very delicate plates, while in the case of a cell with a wide lumen the resin may run down along one side or collect in masses without connection across the cavity. It is not unlikely that the presence of gas bubbles in the cells at this stage may play a part in forming the plates and in determining their location, since in some instances a thin plate may be found at a considerable distance from the ray and without visible connection with it.

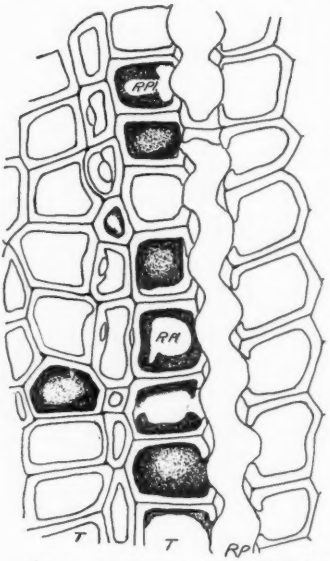

FIG. 1.—Transverse section of *Pinus albicaulis*.

What is the significance of these plates? PENHALLOW says as follows:

> The peculiar form in which the resin is deposited and the particular location of the plates point with much force to their connection with some functional activity, since if it were simply a question of the storage of secreted products, the latter would hardly be disposed as found, but rather after the manner common to so many of the Cupressineae; and this suggestion gains strength from the fact that with respect to the peculiar form of the resin masses as well as their location in the tissue, the Cordaitales are peculiar among the gymnosperms. No exact comparison can be established with other plants, and it is difficult to suggest an adequate explanation. One thing does seem clear, however, and that is that since these plates are of an impervious nature and developed in some cases, at least, in connection with a special constriction of the tracheid cavity, they offer and possibly are specially designed to afford a definite obstruction to circulation in a vertical direction. In this sense they may be designed to serve the same general purpose that is accomplished by the development of tyloses in the vessels of the angiosperms or in the resin passages of the higher Coniferales. It is possible, therefore, that they may be connected in some way, not at present clear, with a more complete restriction of the circulation to a horizontal direction, and particularly through the medium of the medullary rays as specialized channels for that purpose. Among existing

gymnosperms resinous tracheids are almost exclusively confined to *Dammara* and *Araucaria*, though it is a noteworthy fact that similar structures occur rarely among the higher Coniferales. . . . . The taxonomic value of the

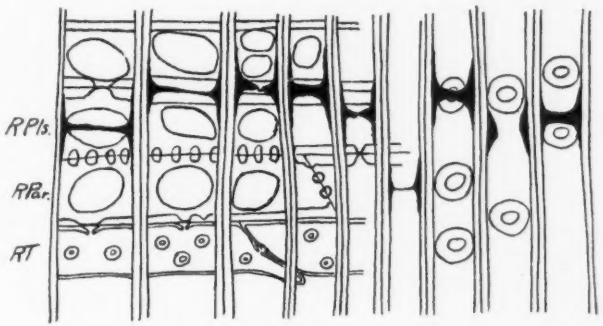

FIG. 2

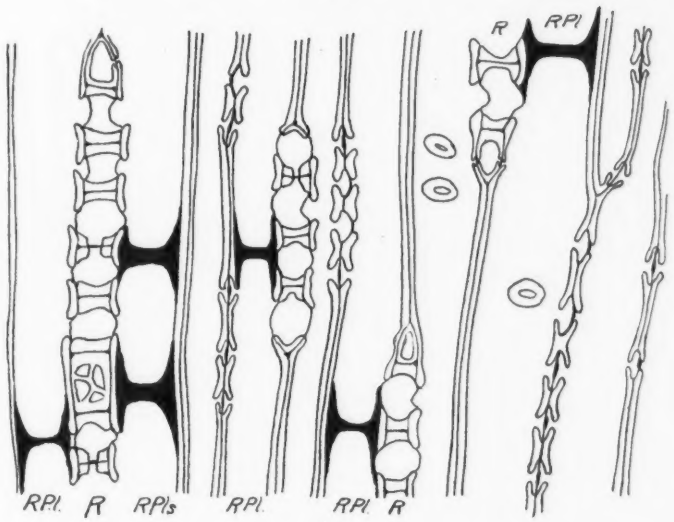

FIG. 3

FIGS. 2, 3.—Fig. 2, radial section; fig. 3, tangential section of *Pinus albicaulis*

resinous tracheids applies exclusively to the Cordaitales, where they are of ordinal value, though in *Dammara* and *Araucaria* they may also become of specific value.

The "peculiar form" and the "particular location," upon which PENHALLOW laid special stress, are readily understood when their origin is appreciated. The resin is produced in the parenchyma as a product, or more likely as a by-product, of the metabolic activity of those cells. The cavities of the adjacent tracheids become reservoirs for such portions of the resin as are excreted. Such excretions may retain the form of globules, or extend across the cavity and assume the form of thick or thin plates, or when the quantity is large nearly fill the cell.

The writer does not consider the "Cordaitales" (*Araucarians*) peculiar among the gymnosperms with respect to the form of the resin masses and their location. The same form and location obtain in *Pinus albicaulis*, not sporadically, but as a constant feature of the heartwood. Similar deposits have also been noted occasionally in the tracheids of *P. resinosa*, *Picea sitchensis*, and in abnormal sapwood of *Pinus ponderosa*, while globules of resin have been observed on the outside of the pit membranes of ray parenchyma cells in *P. Strobus*. These instances, taken in connection with PENHALLOW'S note of the occurrence of resin plates in *P. parviflora* and in two species of *Abies*, lead the writer to believe that they probably occur sporadically in many other representatives of the Coniferae. The writer has also noted tracheids in *P. albicaulis* with resin globules at several lateral pits connecting with the secondary epithelial cells of a vertical resin duct, showing that resinous tracheids in some instances may be independent of the rays.

The association of the resin plates with special constriction of the tracheid cavity, as noted by PENHALLOW and figured by him for *Dammara australis*, appears to the writer to be without special significance. This constriction is due to increase in thickening of the tracheid walls where in contact with the rays, and has been observed by the writer as a common feature of the woods of various genera of the Coniferae, especially in the thick-walled cells formed late in the season. Such increase is presumably due to greater nutrition at that portion, and in most species is not in connection with resin plates, while the resin masses also occur where there are no such constrictions.

PENHALLOW states that no exact comparison can be established with other plants. The writer believes that exact parallels exist in many of the angiosperms. A good example is *Nyssa sylvatica*, and figs. 4 and 5 show resin-like plates (*RPl*) in this wood which exhibit virtually the same origin, form, and distribution as those

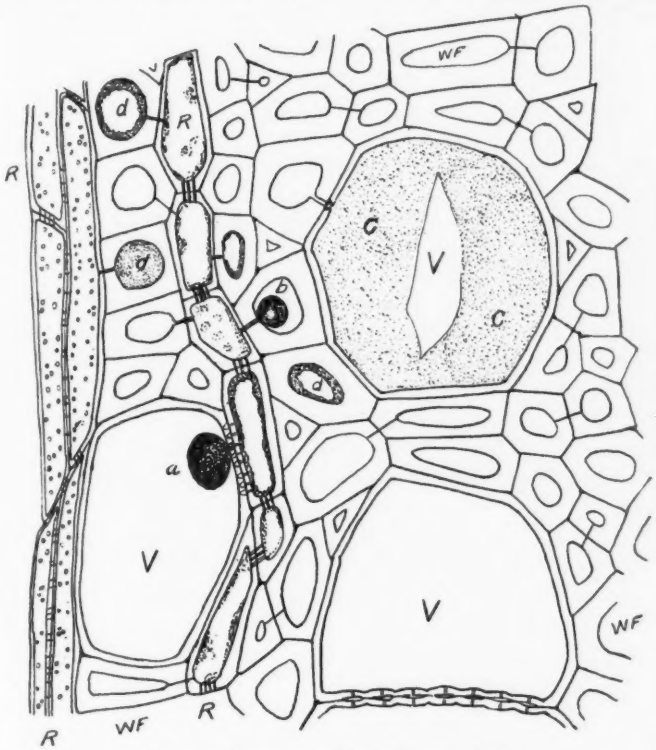

FIG. 4.—Transverse section of *Nyssa sylvatica*

in *Pinus albicaulis*. In *Nyssa* these masses occur in both the tracheae and wood prosenchyma and have their origin in the vertical strands of wood parenchyma (*WP*) and in the rays (*R*). Globules (*a*, *b*) are shown emerging from the pits. The plates across the vessels are thin (*c*), but those in the libriform fibers may be very thick (*d*); in fact much of the fiber cavity may be completely

filled except for occasional bubbles. Seen in transverse section a plate in a vessel appears as a thin imperforate membrane thickened in contact with the wall or with a rupture in the middle (fig. 4, *c*), presumably due to shrinkage. Exactly the same features characterize the resin masses in the tracheids of the gymnosperms.

The presence of secretions (or excretions) in the tracheae only or in both tracheae and prosenchyma in the dicotyledons is very

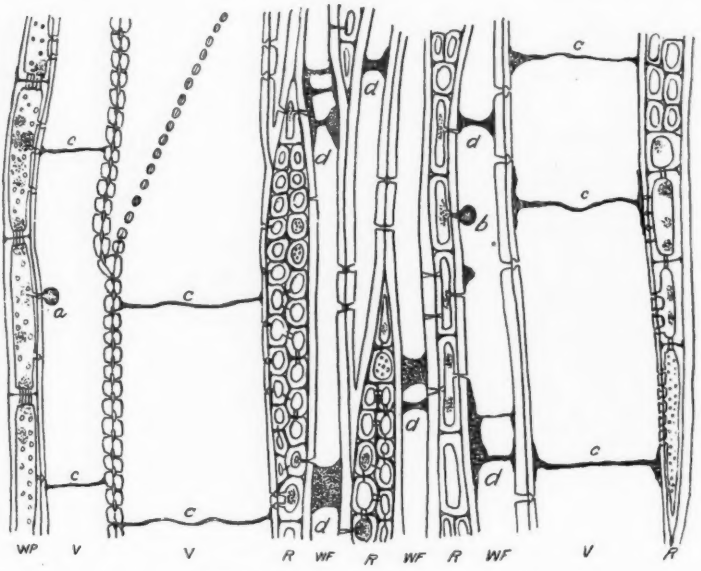

FIG. 5.—Tangential section of *Nyssa sylvatica*

common, and in a great many cases such material is in the form of a collar about the cell wall and with a diaphragm of greater or less thickness across the cavity. The writer believes that these various substances, although different in chemical composition, are alike in being excretions resulting from the metabolic activities of parenchyma cells, and represent waste materials. Although produced in varying amount under normal conditions, the greatest production occurs when the cells are about to cease their vital functions and become heartwood, or when a similar condition is produced abnor-

mally, as in severe wounds. The low vitality of the ray and wood parenchyma at that stage may be considered responsible for the excessive amount of waste matter produced, some of which finds its way or is excreted into the cavities of the adjacent cells.

The parallel between the gymnosperms and angiosperms in the manner of their disposal of secreted or excreted products in the xylem extends further. *Pinus*, *Picea*, *Larix*, and *Pseudotsuga*, for example, have vertical resin canals in the wood. Similar canals are normal to the secondary woods of nearly all genera of Dipterocarpaceae and of *Copaifera*, *Daniellia*, *Eperua*, *Kingiodendron*, *Oxystigma*, *Sindora*, and *Prioria* of the Caesalpineoidae. Vertical resin canals arise traumatically in *Abies*, *Sequoia*, *Tsuga heterophylla*, and others; similarly canals may be produced by injury in *Liquidambar*, *Styrax*, *Terminalia*, *Drimycarpus racemosa*, etc. Resin canals in the medullary rays occur normally in *Pinus*, *Picea*, *Pseudotsuga*, and *Larix;* similar canals have been observed by the writer in representatives of 11 genera of Anacardiaceae and 2 of Araliaceae,[2] and others have reported traumatic canals in both planes in *Liquidambar* and *Styrax*.

The writer concludes that resinous tracheids in gymnosperms find numerous parallels in the angiosperms, that they represent one form of reservoir for excretions, and that the form of the resin masses is in response to well known physical laws. No direct functional activity is attributed to the resin plates, although they are in certain ways analogous to tyloses and reduce the permeability of the wood.

As a diagnostic feature resinous tracheids appear of value in *Pinus albicaulis* and may prove to be so in other cases.

YALE UNIVERSITY

[2] RECORD, SAMUEL J., Intercellular canals in dicotyledonous woods. Jour. Forestry 16:429–441. 1918.

BRIEFER ARTICLES

BISPORANGIATE CONES OF PINUS MONTANA

(WITH ONE FIGURE)

In the latter part of June 1915 the writer found 3 clusters of bisporangiate cones of *Pinus montana* on a tree along the University Drive, Madison, Wisconsin. Nearly all the cones of each cluster bore both macrosporophylls and microsporophylls, the latter being in every case on the lower portion of the cone. The macrosporophylls were borne in most cases on only the upper portion of the cone. In a few instances the cones were almost wholly staminate or pistillate. The sporophylls and spore sacs appeared to be normal in every respect. No abnormalities were observed in the pollen grains which were stained for a microscopical examination. For the past two years the same tree has failed to produce cones of the type described.

FIG. 1.—Cluster of bisporangiate cones of *Pinus montanum;* darker portion pistillate, lighter portion staminate; reduced one-half.

Bisporangiate cones have been reported in only one other species of pine, namely, in *P. maritima* by GOEBEL (1900). However, in a number of other gymnosperms such cones have been described. More than 50 years ago DICKSON (1860) reported them in *Picea excelsa*, later SHAW (1896) in *Sequoia*, and more recently RENNER (1904) in *Juniperus communis*, and HILL and DE FRAINE (1909) in *Pseudotsuga Douglasii*. In every instance, thus far reported, the microsporophylls and macrosporophylls occupied the same relative positions on the cone as in *Pinus montana*.—W. N. STEIL, *University of Wisconsin, Madison, Wis.*

HEALTHY AND SICK SPECIMENS OF BRYOPHYLLUM CALYCINUM

Those who have worked with *Bryophyllum calycinum*, WALKER, DE VRIES, GOEBEL, the writer, and probably many others, have all noticed that the leaves of *Bryophyllum* which form shoots when isolated will rarely or never do so when in connection with a normal and healthy plant. Miss E. L. BRAUN[1] makes the following statement:

> Pot-grown plants of *B. calycinum* in the writer's possession have frequently grown both shoots and roots from leaf notches while the leaves were in connection with the plant. Early in the spring of 1917 a large plant of *Bryophyllum* began to produce shoots from the leaves more abundantly than the plants often do. The accompanying photographs were taken May 12, when shoot production had reached its maximum. It was not necessary to induce the notches to grow; they grew freely under ordinary room conditions, and with only the usual attention which a pot plant in a residence receives.
>
> A number of the leaves of the plant produced shoots from all the notches or from all except the basal notches, a phenomenon which, to accord with LOEB'S theories, should take place only under very special conditions. The plant appears to be a "healthy plant," as healthy and vigorous a plant as the writer has ever seen. Whether or not it is a "normal plant," as a normal plant is conceived of by LOEB, is difficult to say, for nowhere does he define a "normal plant." He does state: "If, however, the flow of substances in a plant is abnormal, either because the roots or the apical parts or both have suffered, a growth of shoots may occur in moist air from the notches of leaves which are in contact with the plant." There is no indication that either the roots or the apical parts have suffered; the plant appears healthy, and has had no accident.

A glance at the photograph accompanying Miss BRAUN'S statement will show to those familiar with "normal" *Bryophyllum* that the plant observed and photographed by Miss BRAUN was a sick specimen. The normal stem of *Bryophyllum calycinum* is perfectly straight and vertical (and unbranched). The specimen observed by Miss BRAUN has not a single straight stem. Stems so weak as not to be able to grow vertically upward are certainly abnormal in regard to nutrition. The bend in the stems acts like a partial block to normal circulation. Such sickly bent stems behave to all purposes like isolated pieces of stems whose leaves will in time give rise to shoots.—JACQUES LOEB, *Rockefeller Institute for Medical Research, New York City.*

[1] BOT. GAZ. **65**: 191. 1918.

CURRENT LITERATURE

NOTES FOR STUDENTS

Hybrid vigor.—The phenomenon of hybrid vigor has come to hold a very important place in practical plant breeding, and is of considerable theoretical interest to geneticists. The most generally accepted interpretation has been EAST'S[1] "heterozygosis," according to which hybrids are vigorous because of their heterozygous sets. Heterozygosis has been very valuable in helping to organize our ideas on the general subject of hybrid vigor, but as a theoretical explanation of the phenomenon involved it has been unsatisfactory. When one says that hybrids are vigorous because of their heterozygous sets, he is making an accurate restatement of the fact of hybrid vigor in the language of genetics, but he is not providing any real explanation of the phenomenon.

The only acceptable "real" explanation that has yet been presented is as follows. In nature a "struggle for existence" occurs among species and individuals. There occurs also a struggle for existence among unit characters. If a unit character is undesirable it is eliminated, since the species possessing it is eliminated. The unit characters, therefore, that have survived and appear in the plants of today are for the most part "desirable" ones, although some undesirable ones also may have survived, having been carried through in association with the "desirable" characters. The majority of unit characters today, however, may certainly be regarded as "desirable" ones, and a majority is sufficient for the present argument.

The question then is raised as to what constitutes a so-called "desirable" character. It may, of course, be any one of a number of things, but is there not some feature common to all such "desirable" characters? The character would seem to be vigor. Each "desirable" character must add somewhat to the vigor of the plant that contains it, and if vigor is increased, such things as size and productiveness will also be increased. Those plants, therefore, will be most vigorous which have in combination the greatest number of "desirable" characters.

The next question is, what plants, in general, have in combination the greatest number of desirable characters? The answer is hybrids, for they combine the "desirable" characters of both parents. Thus, in general, hybrids have twice as many "desirable" characters as do pure races. At this point the objection is raised that though hybrids do actually contain this double quota,

[1] EAST, E. M., and HAYES, H. K., Heterozygosis in evolution and in plant breeding. U.S. Dept. Agric., Bur. Pl. Ind. Bull. no. 243. pp. 58. 1912.

each character is represented by only a single dose in the hybrid and by a double dose in the pure race, so that mathematically the two situations are equivalent. This objection is valid only if we assume complete lack of dominance. We are certainly within our rights in assuming some slight degree of dominance, and if we do this it follows that hybrids have more in the way of active "desirable" characters than have pure races, and, having more "desirable" characters, hybrids are more vigorous. They are vigorous, not because they contain more heterozygous sets, but because they contain more positive dominant characters.

This is a rather obvious explanation of hybrid vigor, and one that has probably occurred to a number of geneticists, being commonly referred to as "the hypothesis of dominance" (accounting for hybrid vigor). It involves 3 assumptions: (1) that there is such a thing as dominance; (2) that most dominant characters are "desirable" ones, that is, of survival value; this assumption is rendered easier if we accept the presence and absence hypothesis; (3) that these dominant "desirable" characters add more vigor than they detract from it, and add to vigor *to the degree in which they are dominant.* This last assumption is the critical one; but even that seems very reasonable.

KEEBLE and PELLEW[2] suggested this explanation in 1910, and since then it has had some discussion in the literature. At first statement the theory seems sound, but actually it does not fit the facts. The two chief objections to this theory of dominance may be found in the publications of SHULL, EMERSON, and EAST (*loc. cit.*).

1. If hybrid vigor were due to dominance, it would be possible in generations subsequent to the F_2 to recombine in one race all of the dominant determiners. Thus there could be isolated a race that was "100 per cent vigorous," and since it would be homozygous, its vigor would not be lost by inbreeding. Actually, though, hybrid vigor cannot be fixed in this way; "all maize varieties lose vigor when inbred."

2. Experience assures us that the distribution of individuals in the F_2 generation with reference to hybrid vigor is represented graphically by a symmetrical curve similar to the normal probabilities curve; the class containing the greatest number of individuals is that which shows the medium amount of hybrid vigor, while on either side of this class the fall in the curve is regular, reaching its lowest point in the two small extreme classes which show respectively greatest hybrid vigor and least hybrid vigor. According to the dominance hypothesis, however, the largest class of F_2 individuals would be that showing the greatest hybrid vigor, while the smallest class would be that showing least hybrid vigor. The curve representing such a situation would be unsymmetrical and strikingly different from that which actually occurs. For these two reasons the dominance hypothesis seems to have been discarded.

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[2] KEEBLE, F., and PELLEW, C., The mode of inheritance of stature and time of flowering of peas (*Pisum sativum*). Jour. Genetics 1:47–56. 1910.

Although it is theoretically attractive, its failure to satisfy these two important details of the hybrid vigor situation has condemned it.

JONES[3] has ingeniously modified the dominance hypothesis so as to avoid these difficulties. At first consideration his theory seems to be clearly the most reasonable explanation of hybrid vigor that has yet been presented, although in time it may encounter destructive criticism. The argument is essentially the same as that for the old dominance hypothesis, with the following important modification. Assume that one parent contains the dominant determiner *A*, linked with the recessive *c;* on another chromosome it contains *B* linked with *d*. The total formula may be expressed conveniently as *Ac*, *Bd*. The other parent has the formula *aC*, *bD*. The hybrid is more vigorous than either parent because it combines all 4 dominant determiners. The attractiveness of this scheme is that it escapes the objections that were made to the older dominance hypothesis: (1) the fact that 100 per cent hybrid vigor cannot be fixed is quite in accordance with JONES' scheme, for it is obviously impossible to isolate a homozygous race, combining the 4 dominant determiners, *A*, *B*, *C*, and *D* (unless crossing over occurs); (2) a simple mathematical demonstration will show that the distribution of F_2 individuals (with respect to hybrid vigor) is quite what it should be, represented by a symmetrical curve, similar to the curve of probabilities. In fact, this new theory, "the dominance of linked factors," seems altogether sound. We should reasonably expect that each chromosome would contain one or more dominant determiners (conducive to vigor) linked with one or more recessives. In this day of factors and determiners such a hypothesis is quite appropriate. It may be, however, that in the future such a phenomenon as hybrid vigor may be explained on the basis of the stabilities and reactivities of the constituents of specific protoplasts.—MERLE C. COULTER.

Taxonomic notes.—BLAKE[4] has published a fascicle of papers containing descriptions of new species. In the paper dealing with Compositae new species are described in *Aphanostephus*, *Diplostephium*, *Verbesina*, *Liabum*, and *Cirsium*. Collections from Venezuela and Curaçao contain new species in the following genera: *Ruprechtia* (2), *Atriplex*, *Bauhinia*, *Croton* (2), *Maytenus*, *Zizyphus*, *Vismia*, *Hecatostemon* (a new genus of Flacourtiaceae), *Passiflora*, *Jacquinia*, *Bumelia*, *Aspidosperma*, *Plumeria*, *Marsdenia* (2), *Lycium*, *Tabebuia*, *Dianthera*, *Oxycarpha* (a new genus of Compositae), *Simsia*, and *Verbesina*. The new species from Oaxaca are referred to *Iresine* (2), *Amyris*, *Guarea*, *Tri-*

[3] JONES, D. F., Dominance of linked factors and heterosis. Genetics **2**:466–479. 1917.

[4] BLAKE, S. F., II. Further new or noteworthy Compositae. Contrib. Gray Herb. N.S. no. 53. pp. 23–30. 1918.

———, New Spermatophytes collected in Venezuela and Curaçao by Messrs. Curran and Haman. *Ibid.* pp. 30–55.

———, New plants from Oaxaca. *Ibid.* pp. 55–65.

chilia, *Comocladia*, *Astronium*, *Myginda*, *Homalium*, *Schismocarpus* (a new genus of Loasaceae), *Cuphea*, *Ardisia*, and *Bouvardia*.

BRITTON[5] has described a new *Scirpus* (*S. Congdoni*) from California, which is the species from the Pacific states heretofore called *S. atrovirens*.

Miss BURLINGHAM[6] has described 4 new species of *Russula* from Massachusetts.

FARWELL[7] has described 17 new varieties of Michigan plants, distributed among 9 families; and has also published a list of rare or interesting plants from the state.

FERNALD[8] has described a new species of *Littorella* (*L. americana*), one of our rarest plants, and heretofore referred to the European *L. uniflora*. The same author,[9] as a result of his study of *Epilobium* from various regions, has published a number of new varieties and combinations and discussed several critical forms.

GREENMAN,[10] in continuation of his monograph of *Senecio*, has presented TOMENTOSI, recognizing 35 species, 2 of which are new, occurring in California and Colorado. The descriptions are accompanied by a full bibliography and liberal citations of exsiccatae, especially such as occur in American herbaria.

JOHNSTON and BRUNER[11] have described a new species of *Phyllachora* (*P. Roystoneae*) found on the leaves of the royal palm (*Roystonea regia*) growing in Cuba. It is described as forming "conspicuous black, carbonaceous masses several centimeters long on the midribs of the leaves."

MACBRIDE[12] has described new species in *Tricyrtis*, *Atriplex*, *Lotus*, *Lomatium*, *Lycium*, and *Cirsium*, and presented the results of his studies of numerous other forms.

MURRILL,[13] in continuation of his studies of the Agaricaceae of tropical North America, has begun the presentation of the subtribe Agaricanae,

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[5] BRITTON, N. L., An undescribed *Scirpus* from California. Torreya **18**:36. *fig. 1*. 1918.

[6] BURLINGHAM, GERTRUDE S., New species of *Russula* from Massachusetts. Mycologia **10**:93–96. 1918.

[7] FARWELL, O. A., New species and varieties from Michigan. Mich. Acad. Sci. Rep. 1917. pp. 247–262.

[8] FERNALD, M. L., The North American *Littorella*. Rhodora **20**:61, 62. 1918.

[9] ———, *Epilobium*, etc. Rhodora **20**:1–10, 29–39. 1918.

[10] GREENMAN, J. M., Monograph of the North and Central American species of the genus *Senecio*. Part II. Ann. Mo. Bot. Gard. **5**:37–108. *pls. 4–6*. 1918.

[11] JOHNSTON, J. R., and BRUNER, S. C., A *Phyllachora* of the royal palm. Mycologia **10**:43, 44. *pl. 2*. 1918.

[12] MACBRIDE, J. FRANCIS, New or otherwise interesting plants, mostly North American Liliaceae and Chenopodiaceae. Contrb. Gray Herb. N.S. no. 53. pp. 1–22. 1918.

[13] MURRILL, WILLIAM A., The Agaricaceae of tropical North America. VII. Mycologia **10**:15–35. 1918.

recognizing 14 species, 6 of which are included in the present contribution. New species are described in *Atylospora* (11), *Psathyrella* (5), *Psilocybe*, and *Campanularius*. In a later paper the same author[14] has described 28 new species from the same region in the following genera: *Drosophila* (8), *Hypholoma*, *Gomphidius*, *Stropharia* (2), *Agaricus* (13), *Coprinus* (4).

MILLSPAUGH and SHERFF[15] have discovered that the species of *Xanthium* are in great confusion, and have described 5 new species, from Vermont (*X. leptocarpum*), New York (*X. arcuatum*), North Carolina (*X. cylindricum*), and Texas (*X. crassifolium* and *X. acutilobum*). In the same paper a new species of *Solidago* (*S. emarginata*) from Illinois is described.

SMITH and SMALL[16] have described a new genus (*Cavea*) of Compositae from India, in the East Himalaya region, belonging to the Inuloideae. It is an extreme alpine form, its structure associating it with *Pluchea*, but its appearance suggesting *Saussurea* or *Berardia*.

STEPHANI,[17] in continuation of his *Species Hepaticarum*, has completed *Metzgeria* and presented 25 other genera, ending with *Plagiochila*. A new genus (*Kormickia*) is described and 121 new species distributed among 9 genera. The largest genus is *Plagiochila* with 187 species, 92 of which are new. The remaining 29 new species are distributed among the following genera: *Metzgeria* (11), *Symphyogyna* (3), *Funicularia*, *Solenostoma*, *Jungermannia* (2), *Jamesoniella* (6), *Anastrophyllum* (3), *Lophozia* (2).

WALTON[18] has described a new genus (*Eutetramorus*) of algae secured from the plankton of a pond on the campus of Ohio State University at Columbus. It belongs to the Coelastraceae (Protococcoideae), the colony consisting of 16 cells.

ZELLER and DODGE[19] have monographed the genus *Rhizopogon* in North America, recognizing 12 species, 6 of which are described as new. In addition, 15 species are presented which have not as yet been found in North America, but may be discovered later. Among these "extra-limital" species 2 are described as new.—J. M. C.

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[14] MURRILL, WILLIAM A., The Agaricaceae of tropical North America. VIII. Mycologia **10**:62–85. 1918.

[15] MILLSPAUGH, C. F., and SHERFF, E. E., New species of *Xanthium* and *Solidago*. Publ. Field Mus. Nat. Hist. **4**:1–7. *pls. 1–6*. 1918.

[16] SMITH, W. W., and SMALL, JAMES, *Cavea*, a new genus of the Compositae from the East Himalaya. Trans. and Proc. Bot. Soc. Edinburgh **27**:119–123. *pl. 5*. 1917.

[17] STEPHANI, FRANZ, Species *Hepaticarum* **6**:49–176. 1917. 1918.

[18] WALTON, L. B., *Eutetramorus globosus*, a new genus and species of algae belonging to the Protococcoidea. Ohio Jour. Sci. **18**:126–128. 1918.

[19] ZELLER, SANFORD M., and DODGE, CARROLL W., *Rhizopogon* in North America. Ann. Mo. Bot. Gard. **5**:1–30. *pls. 1–3*. 1918.

Abscission.—HODGSON[20] and KENDALL[21] have recently contributed to the literature on the abscission problem, the former having investigated foliar abscission in *Citrus* and the latter the abscission of flowers and fruits in 10 genera of the Solanaceae, and particularly in *Nicotiana*. The investigation of an abscission problem may be expected to resolve itself into an effort to determine the following points: (1) the histology of the tissue in which the abscission takes place, and the position of the abscission zone therein; (2) the extent of the abscission zone, its histological differentiation, if any, and its development, that is, whether performed or not; (3) the position of the separation layer within the abscission zone, and the nature of the actual abscission process, that is, the method of cell separation; (4) the time of abscission, involving both reaction time and abscission time; and (5) the possibility of inducing abscission experimentally (by poisonous gases, mechanical injury, etc.). The most vital as well as, often, the most obscure of these matters which should receive consideration is the one involving the determination of the method of cell separation. In this connection both HODGSON and KENDALL found, in the species investigated, that the abscission process conforms to the usual type which involves the separation of cells along the plane of the middle lamella. No cell divisions or elongations were observed to precede or accompany abscission. HODGSON notes a remarkable swelling and gelatinization of the cell walls of the separation layer, which is followed by a dissolution of the gelatinized walls. In this case such cells, after functioning in abscission, resume growth and divide rapidly for a time. The abscission problem in *Citrus* is of peculiar interest because of the well known shedding of immature oranges of the Washington navel variety which annually results in considerable financial loss to orange growers.[22]

KENDALL's article contains a more or less satisfactory consideration of all these points noted as of interest, but, as is perhaps inevitable in an attempt to cover so wide a field, no more than a beginning is made in working out some of the more fundamental problems. Thus, he shows that from water extracts of separation zones in which abscission has commenced a decidedly heavier precipitate comes down in 95 per cent alcohol than from those in which abscission has not started. This difference is tentatively ascribed to the presence of pectin in the first case, it being derived from the hydrolysis of pectose during the dissolution of the primary cell membranes in the activated separation cells. This conclusion may or may not be justified, but such experiments indicate

[20] HODGSON, R. W., An account of the mode of foliar abscission in *Citrus*. Univ. Calif. Publ. Bot. **6**:417–428. 1918.

[21] KENDALL, J. N., Abscission of flowers and fruits in the Solanaceae with special reference to *Nicotiana*. *Ibid.* **5**:347–428. 1918.

[22] HODGSON, R. W., Some abnormal water relations in citrus trees of the arid Southwest and their possible significance. Univ. Calif. Publ. Agr. Sci. **3**:37–54.

lines along which future investigation should lie, especially in view of the fact that KENDALL succeeded with lower percentages of alcohol in bringing down a different type of precipitate. This latter precipitate might be expected to yield cytolytic enzymes. He also finds a reduction in the sugar content of abscission zones following cell separation, and that the normal acidity on *Nicotiana* pedicels is low and is only slightly reduced during abscission. This latter fact is taken to indicate that the activity of enzymes alone is responsible for the dissolution of the middle lamellae during cell separation.

KENDALL reports that illuminating gas and laboratory air will cause abscission in the majority of the species investigated, but that resistance to abscission stimulated in this manner appears suddenly in some species. Tests were also made as to the effect of a variety of mutilations of the flower and pedicel in inducing abscission. Relatively slight injuries to the ovary were effective, whereas considerable amounts of tissue had to be removed in the case of other flower parts before abscission was induced. It is interesting to note that mechanical injury was not found to be particularly effective in the tomato, and that the following species rarely or never exhibit floral abscission: *Nicotiana Bigelovii* (3 varieties), *N. quadrivalvis* (2 varieties), *N. multivalvis*, *Petunia hybrida*, *Salpiglossis sinuata*, *Salpichora rhomboidea*, and *Lycium australis*. A detailed summary of the pertinent literature is included in KENDALL'S paper. —T. H. GOODSPEED.

Nitrates in forest soils and forest regeneration.—In an important contribution HESSELMAN[23] has reviewed the present state of our knowledge of the composition of forest soils and finds, among other things, that while from earth containing relatively little humus it has been possible to isolate organic compounds of known composition the humus of many soils is composed largely of chemical compounds of undetermined character, but that on the whole the constituents are colloidal in nature and are largely influenced by the amount of mineral salts in the soil and ground water. He distinguishes two types of forest humus soils, the "mild humus" characteristic of deciduous forests, well aerated and containing nitrate-forming as well as denitrifying bacteria, and "raw humus" found in coniferous forests as a series of layers of leaves and litter in various stages of decomposition from which nitrate-forming and denitrifying bacteria are usually absent.

Recognizing decomposing litter as one of the principal sources of nitrogen in forest soils, he has investigated the "decay capacity" of various forest types, using several different methods. He has determined the relative abundance of various bacteria, the nitrogen content of trees and plants, and has shown that nitrate supply and nitrate formation is at its maximum in beech forests and at its minimum in mossy coniferous stands. Lime in the soil and in solution

[23] HESSELMAN, HENRIK, Studier över saltpeterbildningen i naturliga jordmåner och dess betydelse i växteekologiskt avseende (with abstract in German). Meddel. från Statens Skogsförsöksanst. Haft. 13–14. 297–527. *pls.* 7. *figs.* 30. 1917.

in the ground water tends to promote nitrification. He points out that by proper forest management the formation of nitrates may be accelerated and a decided increase in timber production obtained.

In a second article[24] he investigates the problems of the regeneration of conifer forests, with particular reference to the transformation of nitrogen, for it appears that while trees of pine and spruce often grow in forests where no nitrate formation is taking place, the raw humus developed beneath their dense shade does not prove a good soil for the rapid growth of their seedlings. It seems from experimental evidence that nitrogen transformation in such soils may be initiated and accelerated by the introduction of light through cutting, by burning the surface, or by stirring the surface soil. Decaying timber seems to favor nitrogen transformation, and this may tend to account for the observed abundance of conifer seedlings growing upon fallen logs.

In mixed conifer stands, especially where the herbaceous undergrowth is good, nitrate formation is, in contrast, rather active; so much so in many instances as to induce such a rank growth of herb and grass vegetation in clearings as to crowd out conifer seedlings. These and other data should help to explain to the ecologist many phenomena of secondary succession, while from the same data the forester should receive guidance for the formulation of a policy of forest management that will favor the formation of the amount of nitrogen best suited to the regeneration of the forest.

The value of these excellent papers is increased by an abundance of tabulated data, by being freely illustrated, and by extensive bibliographies.—GEO. D. FULLER.

Mechanics of movement in insectivorous plants.—Two recent papers on this subject, by BROWN[25] and by HOOKER,[26] have supplied some interesting information. Although different plants were used, the results are comparable in many respects. Both investigators find that the bending is accompanied by an extension of the cells on the convex side, which soon becomes fixed by growth; that there is little or no change of size in the cells of the concave side; and that unbending is accompanied by growth on the concave side. HOOKER finds the osmotic pressure of the cells on the convex side of bending tentacles less than that on the concave side, and this decrease is proportional to the increase in the length of the cells. He finds no changes in permeability and concludes that the increased size of the cells is due to decreased elasticity of the cell walls.

[24] HESSELMAN, HENRIK, Om våra skogsföryngringsåtgärders inverkan på saltpeterbildningen i marken och dess betydelse för barrskogens föryngring (with abstract in English). Meddel. från Statens Skrogsforsöksanst. Haft 13–14. 923–1076. *pls. 15. figs. 48.* 1917.

[25] BROWN, WM. H., The mechanism of movement and the duration of the effect of stimulation in the leaves of *Dionaea*. Amer. Jour. Bot. **3**:68–90. 1916.

[26] HOOKER, HENRY D., JR., Mechanics of movement in *Drosera rotundifolia*. Bull. Torr. Bot. Club **44**:389–403. 1917.

BROWN reports no determinations of osmotic pressure, but finds that if closed leaves of *Dionaea* are killed, before the extension of the cells has become fixed, and passed through alcohol to xylene, the leaves reopen, and close again when passed back through alcohol to water. He concludes that the increase in size of the cells is due to increased osmotic pressure. He believes there is no permeability change, and thinks changes in the elasticity of the cell walls improbable. It is interesting if, in fact, the mechanics of these two responses, so similar in many respects, are so widely different in another.

Geotropic bending of growing organs is similar in many respects to the movements studied. Its comparative slowness should make it somewhat easier to follow, and the results might furnish valuable suggestions as to the mechanics of these more rapid movements. SMALL[27] has found differences in permeability in the two flanks of *Vicia Faba*, roots bending geotropically.—THOMAS G. PHILLIPS.

Soil moisture studies.—The extensive investigations of BRIGGS and SHANTZ have shown the importance of the moisture equivalent as a constant that will measure the physical properties of soils. Two recent studies deal with certain phases of the same phenomena. The first[28] shows that while the addition of various salts does not materially change the moisture equivalent of the soil under investigation, if the same salts are washed from the soil with water it then seems to possess a new and peculiar set of physical properties and its moisture equivalent is markedly increased. This increase varies from 2 to 40 per cent, and is taken to mean that the washing out of the salt has increased the interior surface of the soil.

The second article, by SMITH,[29] reports the investigation of the relationship between the results of mechanical analysis and the moisture equivalent. He concludes that there is at present no formula that gives more than a rough approximation of this relationship, and hence that the moisture equivalent cannot be indirectly determined by mechanical analysis with any degree of accuracy.—GEO. D. FULLER.

Soil aeration and root growth.—Roots of various plants appear, according to the results of CANNON and FREE,[30] to respond quite differently to variations in the composition of the soil atmosphere, and this difference in response seems

[27] SMALL, JAMES, Geotropism and the Weber-Fechner law. Ann. Botany **31**:313–314. 1917.

[28] SHARP, L. T., and WAYNICK, D. D., The moisture equivalent determinations of salt-treated soils and their relation to changes in the interior surfaces. Soil Sci. **4**:463–469. 1917.

[29] SMITH, ALFRED, Relation of the mechanical analysis to the moisture equivalent of soils. Soil Sci. **4**:471–476. 1917.

[30] CANNON, W. A., and FREE, E. E., The ecological significance of soil aeration. Science, N.S. **45**:178–180. 1917.

to be related to the character of the natural habitat of the species in question. Thus *Salix* sp. (probably *nigra*) stands at one end of the series and shows no injurious effect even when the oxygen of the atmosphere is entirely replaced by either nitrogen or carbon dioxide. At the opposite end of the series stands *Opuntia versicolor*, growth of roots ceasing with an atmosphere containing 50 per cent carbon dioxide, while *Coleus Blumei* is comparable to it, showing injury and ultimate death with the addition of 25 per cent nitrogen to the soil atmosphere. Of the other species tested *Heliotropium peruvianum* was closely comparable to *Opuntia*, while *Nerium oleander* and *Prosopis velutina* prove nearly as resistant as *Salix*. The results seem to indicate that plants growing naturally in well drained soil are much more sensitive to the composition of the soil atmosphere than those from swamps and poorly drained habitats.—GEO. D. FULLER.

Embryo of Aucuba.—PALM and RUTGERS[31] have settled the question of apogamy in *Aucuba japonica*, which has been under suspicion for 40 years. They bagged 300 pistillate flowers and not a single fruit formed, while 600 isolated pistillate flowers produced normal fruit after artificial pollination. It is thought that EICHLER's original suggestion of apogamy probably came from the fruiting of an isolated pistillate plant which had developed staminate flowers, since the authors have repeatedly found staminate flowers on pistillate plants. Staminate plants have also been observed to produce pistillate flowers.

The flowers open about the time of megaspore formation, and the embryo sac reaches the fertilization stage about 4 weeks later. The solitary megaspore mother cell becomes deeply placed by the extensive development of parietal tissue. The behavior of the 4 megaspores is usually quite normal, but in one case the 2 megaspores nearest the chalaza were found in division. The development of the gametophyte is normal, but stages in endosperm formation were not obtained. The chromosome numbers were determined to be 18 and 36. —J. M. C.

Disease resistance.—JONES[32] has published a summary of his results in securing a race of cabbage resistant to the "yellows." Some of the fundamental questions involved in resistance were considered. The difference between susceptible and resistant plants was found not to be due to any superficial obstacle, but to the different relations of the interior cells of the host and parasite. "The resistant tissues have the ability to restrain the development of the parasite to a greater degree than do the susceptible and so give time for protective cork formation." It was shown also that resistance is clearly inheritable, not as a single character, but as a complex of a number of heritable

[31] PALM, BJ., and RUTGERS, A. A. L., The embryology of *Aucuba japonica*. Rec. Trav. Bot. Néerland. **14**:119–126. *figs. 12*. 1917.

[32] JONES, L. R., Disease resistance in cabbage. Proc. Nat. Acad. Sci. 4:42–46. 1918.

factors. Environmental factors were found to have a marked influence upon invasion by the parasite (*Fusarium*), there being a "critical soil temperature" (about 17° C.) for such invasion. Below this the plants are not invaded even in the sickest soils.—J. M. C.

Desiccation.—An investigation of the course of desiccation and partial starvation in cacti has been made by MACDOUGAL, LONG, and BROWN.[33] The principal studies center upon the changing rate of water loss, chemical changes in the food reserves, plasmatic colloids and cell sap, and the morphological changes which occur during long periods of desiccation. In one case a large *Echinocactus* was under observation for 6 years after removal of the plant from the soil. Water loss is rather rapid at first, but proceeds more and more slowly with time. While 10 per cent of the water was lost the first year in one specimen, during the sixth year only 5 per cent of the water remaining at the beginning of that year was lost. The loss of water is much more rapid of course in the open than in diffuse light, and *Echinocactus* can withstand desiccation not more than 2 years with free exposure.—GEO. D. FULLER.

Aeration of nutrient solutions.—STILES and JÖRGENSEN[34] find that aeration of the nutrient solution increases the rate of growth of barley, as found by various workers, but has no effect on the growth of buckwheat, as found by FREE. They carefully limit their conclusion to the condition under which they experimented, and find themselves unable to explain this specific difference. They emphasize the necessity of knowing much more about the physical chemistry of water culture solutions. They also feel that neither the law of the minimum nor the principle of limiting factors gives an adequate expression of the behavior of the plant as a whole.—WM. CROCKER.

Apogamy in ferns.—STEIL[35] has discovered apogamy in a large number of ferns, the investigation extending over a period of 6 years. It seems that apogamy is of frequent occurrence in *Pellaea*, *Pteris*, and *Aspidium*. The prothallia were grown under cultural conditions favorable for the development of sex organs and embryos in non-apogamous species. Many interesting details of embryo development are given, which much extend our knowledge of this phenomenon.—J. M. C.

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[33] MACDOUGAL, D. T., LONG, E. R., and BROWN, J. G., End results of desiccation and respiration in succulent plants. Physiol. Res. 1:289–325. 1915.

[34] STILES, W., and JÖRGENSEN, I., Observations on the influence of aeration of the nutrient solution in water culture experiments, with some remarks on the water culture methods. New Phytol. 16:182–197. 1917.

[35] STEIL, W. N., Studies of some new cases of apogamy in ferns. Bull. Torr. Bot. Club 45:93–108. *pls. 4, 5.* 1918.